Nitrate-nitrogen effects on benthic invertebrate communities in streams of the Canterbury Plains

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Abstract

Aquatic ecosystems are especially vulnerable to human impacts associated with agricultural land-use, which provide multiple stressors altering community composition, important ecosystem functions and human valued properties of freshwaters. However, the increased occurrence of excessive levels of nitrate-nitrogen has raised major concerns about toxicity and stress on aquatic life, especially in regions such as the Canterbury Plains, New Zealand. The aims of this thesis were to identify nitrate-nitrogen effects on stream communities, and additionally provide field data to inform proposed national bottom lines for nutrients in New Zealand streams. A field survey was conducted on 41 small streams on the Canterbury Plains spanning a nitrate-nitrogen gradient (mean 0.4 – 11.3 mg/L). Spot nitrate-nitrogen was collected during and after the field survey to measure temporal variation in stream nitrate-nitrogen concentration for six months. This showed nitrate-nitrogen concentration varied between season and sub-region, where concentrations increased in winter and Ashburton had higher nitrate-nitrogen than Rangiora and Lincoln, respectively. These regimes of nitrate-nitrogen showed similar patterns in mean, median and maximum concentrations. To be confident my spot nitrate-nitrogen provided a true representation of long-term water chemistry, I compared Environment Canterbury 12 monthly data with my six monthly data in a sub-set of 15 sites. This comparison showed similar nitrate-nitrogen patterns and range of values between the two datasets. I then compared 12 common benthic invertebrate biotic metrics with my nitrate-nitrogen data and found none were correlated with this contaminant. For example, the Macroinvertebrate Community Index and quantitative variant (QMCI) derived to measure the response to organic pollution provided inconsistent results when applied to my streams. Nevertheless, gut content stoichiometry of the common mayfly grazer *Deleatidium* spp. indicated improvement in food quality (lower C:N ratio) with higher nitrate-nitrogen concentrations. These results indicated either nitrate-nitrogen does not alter invertebrate structural metrics across this nitrate-nitrogen gradient, or that these biotic metrics measure community structure aspects not affected by

nitrate-nitrogen. I then investigated possible community composition patterns across the nitrate-nitrogen gradient. Unconstrained ordination (on presence/absence data) showed invertebrate communities at my sites were influenced primarily by discharge and shade, with the next most important driver being nitrate-nitrogen. A constrained ordination (on the same data) testing the singular effect of nitrate-nitrogen showed a marginally non-significant change in composition, with higher variability in community composition at higher nitrate-nitrogen concentrations. A further aim of my study was to test the draft nitrate-nitrogen bands proposed by Hickey (2013). These nitrate-nitrogen bands may advise guidelines to protect aquatic organisms as required by the National Policy Statement on Freshwater. Analysis of my invertebrate communities showed differences in composition, particularly at < 1 and > 6.9 mg/L bands. Several predatory caddisfly taxa: *Triplectides*, *Neurochorema* and *Oeconesus* were identified as potential indicator species of communities associated with low nitrate-nitrogen. These findings show that nitrate-nitrogen effects are difficult to detect, and that it is not the main driver of community composition in Canterbury streams. However, nitrate-nitrogen may be an important stressor for sensitive benthic invertebrate communities, as effects were observed on pollution tolerant organisms in this study. Therefore, this research has implications for freshwater ecologists and environmental managers striving to improve the health of streams on the Canterbury Plains.

Chapter 1: Introduction

Globally, ecosystems are experiencing increasing stress due to human impacts, including land-use conversion to farmland, faster cycling of nutrients, climate change warming, loss of biodiversity and invasions (Vitousek et al. 1997; Sala et al. 2000). Understanding how human activities impose these stressors on biological community structure, functional processes and valued ecosystem services is a major challenge for ecologists (Carpenter et al. 1998; Foley et al. 2005). Freshwater ecosystems, which are highly valued for economic, recreational, cultural, aesthetic, scientific and educational properties, are especially vulnerable to human impacts as represent the condition of their surrounding landscapes. These impacts include the over-exploitation of freshwater resources, water pollution, flow modification, habitat degradation and species invasion (Sala et al. 2000; Dudgeon et al. 2006). One of the most problematic human impacts which has accelerated in the past two decades has been the alteration of the global nitrogen cycle; excessive inputs of nitrogen pollute waterways, which impose stress on freshwater ecosystems (Tilman 1998; Camargo, Alonso & Salamanca 2005; Camargo & Alonso 2006). To address impacts of elevated nutrients on ecosystem properties of biodiversity, ecosystem functions and community resilience, the clear communication of ecological knowledge between scientists and environmental managers is required (Anderson & Bows 2012). In this thesis I investigate if increasing nitrogen concentrations (measured as nitrate-nitrogen) effect freshwater benthic macroinvertebrate communities in the Canterbury Plains of New Zealand.

Human inputs of nitrogen, predominantly in the form of nitrate-nitrogen have increased substantially by advances in the ‘green revolution’ through application of fertiliser to increase crop yields (Khush 2001). Nitrate-nitrogen enters freshwater ecosystems when the capacity for crops to up-take nutrients in the

agricultural landscape is exceeded, whereupon nitrate-nitrogen becomes potentially available for transport (Carpenter et al. 1998). This has a number of ecological consequences such as altered freshwater ecosystem productivity and dynamics (Carpenter et al. 1998; McDowell, Larned & Houlbrooke 2009). Nitrate-nitrogen can be a limiting nutrient in freshwater ecosystems, however excessive nitrogen may result in toxic algal blooms, anoxia, fish kills and depletion of biodiversity (Carpenter et al. 1998; Hughey et al. 2013). Given that application of nitrate-nitrogen fertilisers is now equal to all natural inputs into the global nitrogen cycle (Tilman 1998), and future fertiliser application is predicted to increase (Galloway & Cowling 2002), the effects of nitrate-nitrogen on aquatic systems is a pressing concern for the Canterbury region and similar agricultural areas worldwide. Therefore, research on the effects of nitrate-nitrogen on freshwaters of the Canterbury Plains is timely, and also imperative for the conservation of resources in New Zealand.

Nitrate-nitrogen issue on the Canterbury Plains

The agricultural history of New Zealand is unusual, having undergone agricultural development over a relatively short period of time (approx. 150 years) compared to agriculturally-intensified regions in the northern hemisphere (Didham, Denmead & Deakin 2012; Greenwood et al. 2012). The Canterbury Plains has experienced substantial agricultural intensification within the past 30 years, largely due to the increase in dairy farming land-use from 20,000 to 282,000ha between 1980 and 2012 (Pangborn & Woodford 2011; Hill 2013). This rapid increase has been driven by a market for high outputs of dairy production at low cost (PCE 2004). The Canterbury Plains has had the greatest increase of cows per hectare (~ 1.8 to 3.2 cows/ha between 1982/83 - 2007/08) and production of milk-solids per cow (~ 240 to 375 kg/cow between 1982/83 - 2007/08) in New Zealand (Pangborn & Woodford 2011). Attaining this level of dairy intensification has exponentially increased use of water and energy resources, as well as pesticides, imported feed-stock and fertilsers (Didham et al. 2012). Thus, dairying has been the primary driver of land-use intensification and environmental impacts in Canterbury and New Zealand in the past 20 years (PCE 2004; MacLeod & Moller 2006). Nitrate-nitrogen inputs are a strong indicator of historical and present agricultural practices in the

Canterbury Plains and, with the predicted doubling in land area for dairy farming in the next 20 years, nitrate-nitrogen concentrations will likely continue to rise in freshwaters (Pangborn & Woodford 2011).

The National River Water Quality Network (NRWQN) includes water quality parameters from 77 sites throughout New Zealand and covers waters which drain approximately half of the nations landscapes since 1989 (Ballantine & Davies-Colley 2010). The NRWQN documented increasing oxidized nitrogen (measured as nitrate-nitrogen + nitrite-nitrogen) concentrations at the national level between 1989 and 2007 (Ballantine & Davies-Colley 2010). Within the Canterbury region, spring fed-streams recorded median dissolved inorganic nitrogen (dominantly nitrate-nitrogen over nitrite-nitrogen) concentration of 1.8 mg/L between 2002 and 2007 (Stevenson, Wilks & Hayward 2010). Coastal areas of the Canterbury Plains (between the Rangitata and Rakaia river systems) had a particularly high median nitrate-nitrogen concentration of 5.6mg/L from 2000 – 2005 (Meredith et al. 2006). The high nitrate-nitrogen concentrations found in spring-fed streams have been shown to be partially due to nitrate-nitrogen contaminated groundwater (Stevenson et al. 2010). In fact, the Ministry of Health maximum acceptable value (MAV) of 11.3mg/L of nitrate-nitrogen, set to protect drinking water for human consumption, was exceeded in 8.7% of Canterbury groundwater wells sampled in 2012; values ranged from < 0.1 – 64 mg/L with a median value of 4.2 mg/L (Wong & Hanson 2012; Ministry of Health 2013). Furthermore, while nitrate-nitrogen concentrations are elevated in the Canterbury Plains, discrepancies between concentrations of groundwater and spring-fed streams suggests there may be a lag period in nitrate-nitrogen transport (Stevenson et al. 2010). Therefore, the effects of historical land-use practices contributing to nitrate-nitrogen inputs may not be currently observed in Canterbury surface waters.

Recognition of nitrate-nitrogen as an important freshwater containment in New Zealand has prompted management strategies to work towards reducing nitrate-nitrogen concentrations. These strategies include supporting best management practices to reduce leaching (Francis 2005; Ford & Taylor 2006), inhibiting transport from the agricultural landscape by application of dicyandiamide (DCD) onto soils (Francis 1995; Di,

Cameron & Sherlock 2007), and proposing nitrate-nitrogen thresholds to protect human health (Ministry of Health 2013) and aquatic organisms (Hickey & Martin 2009; Hickey 2013). For the protection of aquatic organisms, the National Policy Statement on Freshwater (NPS-FW) requires nitrate-nitrogen guidelines to be implemented in New Zealand freshwater ecosystems (Hickey 2013). However, the values proposed in such guidelines are based on ecotoxological trials and thus are subject to some limitations when applied to freshwater ecosystems. For example, laboratory experiments can rarely adequately represent potentially complex whole community responses to nitrate-nitrogen, as well as to identify more subtle species-specific reductions in fitness/physiological responses, or detect feedbacks within a system. Therefore, although ecotoxological trials may be used to decide a range in which nitrate-nitrogen may be a stressor, the NPS-FW guidelines would be substantially strengthened by the inclusion of empirical evidence based on field data. A goal of this thesis was to generate field data to inform decisions on appropriate limits of nitrate-nitrogen for aquatic organisms. These limits are currently in development to form water quality national bottom lines for New Zealand (Ministry for the Environment 2013).

Transport mechanisms and stress pathways on aquatic life

Nitrate-nitrogen in freshwater ecosystems comes from both natural and anthropogenic point and non-point inputs via transformation of nitrogen (Figure 1)(Ford & Taylor 2006). In natural systems dissolved inorganic nitrogen concentrations are low (0.12mg/L) with nitrate-nitrogen the major form (0.1 mg/L) compared with nitrite-nitrogen (0.001mg/L) and ammonium-nitrogen (0.015mg/L) (Allan & Castillo 2007). Natural sources include atmospheric deposition, nitrogen rich geological deposits, certain prokaryotes (i.e. cyanobacteria) and organic matter decomposition (Camargo et al. 2005). Anthropogenic inputs include agricultural and urban runoff and discharges from industrial waste, sewage effluent, offal pits and fertiliser (Carpenter et al. 1998; Camargo et al. 2005; Ford & Taylor 2006). In Canterbury, total non-point pollution accounts for 90% of nitrogen losses (Ford & Taylor 2006). Nitrogen can be transported into surface waterways by leaching, subsurface flow from tile drains, and associated with sediment erosion and soil release (Carpenter et al. 1998). An estimated total of 20 million kg of nitrate-nitrogen is leached per year in the Canterbury Region

(Dymond et al. 2013). This is among the highest total nitrate-nitrogen leaching in New Zealand, alongside the Waikato and Manawatu-Wanganui regions, which leach approximately 35 million and 20 million kg of nitrate-nitrogen per year, respectively (Dymond et al. 2013). However, the Waikato and Manawatu-Wanganui regions have maintained similar leaching rates over the past 20 years, whereas total nitrate-nitrogen leaching in Canterbury has doubled during the same period (Dymond et al. 2013). This indicates that sources of nitrate-nitrogen available for transport have increased, which is a major concern for groundwater contamination, as groundwater provides 80% of water storage in the Canterbury Plains (Ford & Taylor 2006).

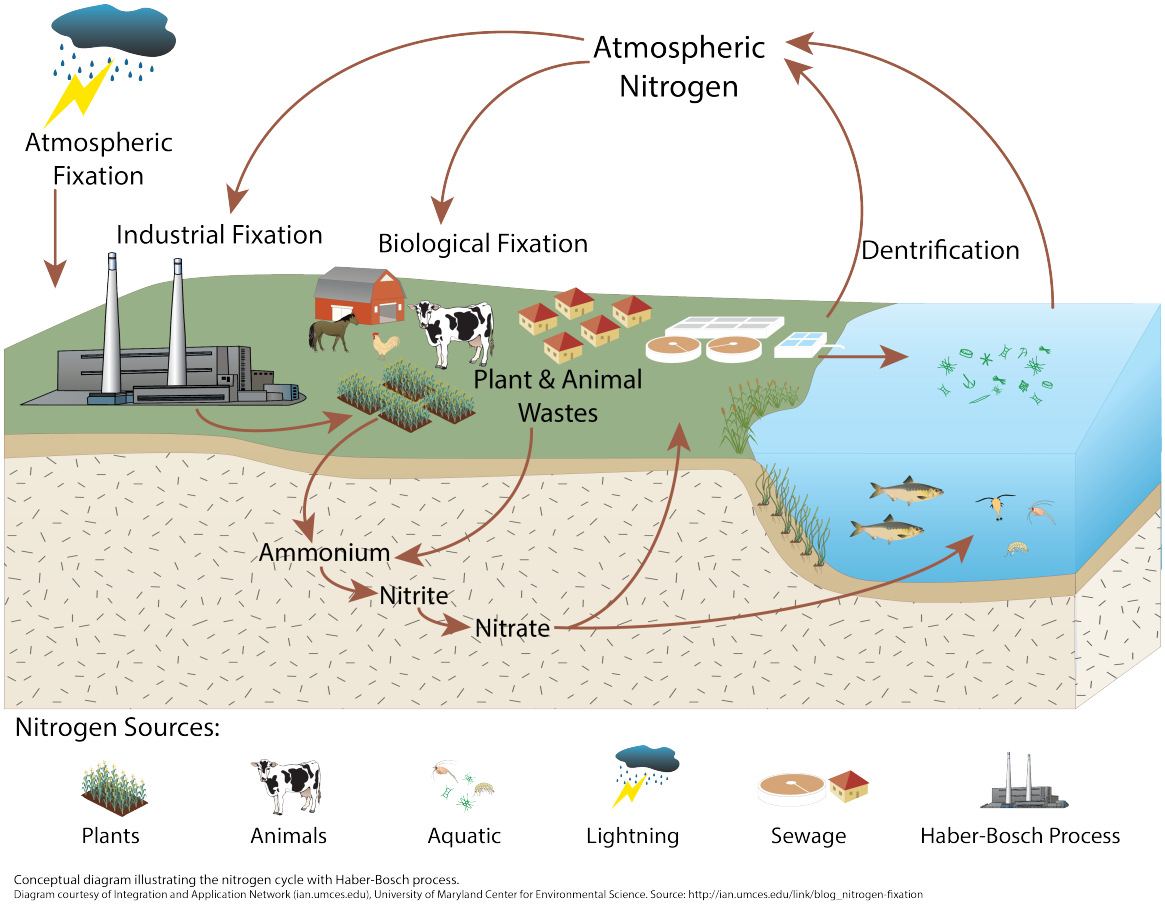

Figure 1: Nitrogen cycle reproduced from the Integration and Application Network, University of Maryland Center for Environmental Science (Ward 2013). Arrows indicate transformation pathways of nitrogen.

Among the most essential chemical constituents of living organisms in freshwater ecosystems are nitrogen (N) and phosphorus (P), primarily as nitrate-nitrogen and dissolved reactive phosphorus (Allan & Castillo 2007; Dymond et al. 2013). Consequently demand for N and P limit community dynamics, most notably growth of algae productivity (Heathwaite, Sharpley & Gburek 2000; Allan & Castillo 2007). If algal growth is not limited by the availability of N and P, prolific algal growth often occurs, resulting in algal blooms that can have detrimental effects on aquatic ecosystems (Biggs 2000). This process is termed eutrophication, and provides an indirect pathway by which nitrate-nitrogen affects freshwater communities (Vitousek et al. 1997). Alternatively, nitrate-nitrogen can have a direct effect on aquatic organisms due to conversion of oxygen carrying pigments (e.g. hemoglobin) into forms that cannot carry oxygen (e.g. methemoglobin) (Camargo et al. 2005). While the nitrate ion is usually considered to have low toxicity due to its low bronchial permeability, it can be converted into other more toxic forms, such as nitrite or ammonia, through processes of the N cycle (Allan & Castillo 2007). Both direct and indirect pathways of nitrate-nitrogen can operate simultaneously to alter community composition of benthic macroinvertebrates in the freshwater ecosystem.

Relationship between nitrate-nitrogen and aquatic structure and function

Overseas, assessment of nitrate-nitrogen effects on macroinvertebrates has been used to identify ecological indicators and nutrient thresholds to support nutrient criteria development (Wang, Robertson & Garrison 2007; Yuan 2010; Chambers et al. 2012). A regional scale survey recorded rising nutrients decreased invertebrate richness in open-canopied streams in the United States of America (Yuan 2010). These declines were not indirectly mediated by changes in periphyton biomass (Yuan 2010). In Wisconsin, a field survey sampling 240 streams spanning a range of phosphorus (0.012 – 1.64 mg/L) and nitrogen (0.13 – 21.26 mg/L) concentrations documented 69% of macroinvertebrate indicators (e.g. percent EPT and taxa) were significantly correlated with at least one nutrient (Wang et al. 2007). Additionally, of the selected environmental factors explaining variation in macroinvertebrate communities, 22% was attributed to nutrients (Wang et al. 2007). A Canadian survey, analyzing > 200 long-term monitoring stations, showed increase in nutrients was associated with high benthic algal abundance and loss of sensitive benthic

macroinvertebrate taxa (Chambers et al. 2012). Using this information, N thresholds were derived, ranging from 0.21 – 1.2 mg/L between different regions of Canada (Chambers et al. 2012).

In New Zealand, Clapcott et al (2012) showed that nitrate-nitrogen + nitrite-nitrogen concentrations were different between native vegetation, urban and agricultural landscapes. At a threshold of 0.316 mg/L of nitrogen a change in the response curve for water quality, ecosystem processes and invertebrate indicators was documented, indicating a potential limit to protect ecological integrity of stream systems (Clapcott et al. 2012). Similarly, by using predicted nitrogen concentration as an indicator of land-use change, a comparison with five functional indicators of stream health (gross primary productivity, ecosystem respiration, cellulose decomposition potential, wooden stick breakdown and $\delta^{15}N$ of primary consumers) documented a change in relationship threshold at 0.5 and 3.2 mg/L of nitrogen (Clapcott et al. 2010). Structural indicators of land-use have observed shifts in benthic macroinvertebrate communities from native forest and tussock grassland to agricultural pasture in New Zealand (Quinn & Hickey 1990; Hall, Closs & Riley 2001). Sensitive Ephemeroptera, Plecoptera and Trichoptera taxa frequently decline while Oligochaeta, Chironomidae and the snail *Potamopyrgus antipodarum* increase with agriculture (Quinn & Hickey 1990; Hall et al. 2001). However, changes in benthic macroinvertebrate communities with land-use can only be indirectly attributed to increased nitrate-nitrogen concentrations (Hall et al. 2001).

Nitrate-nitrogen may affect structural aspects of the benthic macroinvertebrate communities in predictable ways. For example, the benthic macroinvertebrate sensitivity index (MCI) declined with increasing nitrate-nitrogen in Manawatu streams (Death, unpublished data). Similarly, declines in snail and insect invertebrate groups and increases in ostracods have also been related indirectly to nitrate-nitrogen in the highly enriched Waipara River (Suren et al. 2003). Furthermore, in Southland streams benthic macroinvertebrate richness (grazers and predators) was negatively associated with N concentrations (Liess et al. 2012). A unimodel relationship is often predicted, as nutrient enrichment can form a subsidy-stress effect (Niyogi et al. 2007b). In this scenario a small quantity of nutrients stimulates primary productivity, thus benefitting community

composition and/or biodiversity, but excessive nutrients could cause algal blooms, anoxic conditions and reduction of habitat quality resulting in stress and lower biodiversity (Niyogi et al. 2007b). A study on 21 Otago streams found support for a unimodel subsidy-stress ecological response (EPT density and richness, MCI, percent non-insect taxa) across a gradient of nitrate-nitrogen from 0 – 1.8mg/L (Niyogi et al. 2007b). Similarly, investigation of 18 Otago headwater pasture streams found higher richness and density of benthic macroinvertebrates with slightly elevated nitrate-nitrogen of 0.03 mg/L, although no stress was observed with increasing nitrate-nitrogen possibly due to low concentrations (Riley et al. 2003).

Nitrate-nitrogen has also been predicted to have an additive stress on freshwater communities in combination with other stressors, such as sediment and low flows (Wagenhoff et al. 2011; Piggott et al. 2012). Matthaei et al (2010) used stream mesocosms with three concentrations of N and P nutrients (nitrate-nitrogen natural ~ 0.03 mg/L, intermediate ~ 0.1 mg/L and high ~ 0.56 mg/L) with 3 sediment levels (natural ~ 1% cover, intermediate ~ 3% cover, high ~ 4% cover) and 2 flow levels (control ~ 2.1 Ls^{-1} and reduced ~ 0.43 Ls^{-1}), to test for singular and interactive stressor effects. Nutrient enrichment had a singular effect on invertebrate abundance, algal biomass and leaf decay rates, all of which increased, indicating a subsidy response (Matthaei, Piggott & Townsend 2010). There was an interactive effect between flow and nutrients; reduction in flow reduced abundance of certain taxa. Sedimentation effects were increased at high nutrients, also indicating an interaction between sediment and nutrients (Matthaei et al. 2010). In a similar experiment conducted in 128-stream mesocosms in which N and P nutrients (~ 0 – 6.8 mg/L nitrate-nitrogen) and sediment (0 – 100% cover) were manipulated, nutrient enrichment initially increased EPT density, but N concentrations over after 0.7 mg/L resulted in a negative effect (Wagenhoff, Townsend & Matthaei 2012). A synergistic effect was also found between nutrients and sediment; strength of the negative interactions was higher at high levels of both stressors.

Thesis structure and aims

This thesis was presented in that the aims of each chapter are based on questions explored in the previous chapters. The format is intended to reduce repetition of methodologies and introductory information as well as to represent the exploratory nature of this research. The aim of this thesis was to investigate if nitrate-nitrogen affects benthic invertebrate communities in streams of the Canterbury Plains.

Chapter One gives context and background information explaining the global context and specific nitrate-nitrogen issue in the Canterbury Plains. Chapter Two documents a field survey conducted on the Canterbury Plains to study the response of benthic macroinvertebrate communities across a gradient of nitrate-nitrogen. In this chapter I investigated the benthic macroinvertebrate relationship with metrics commonly used to assess water quality by environmental managers. This approach compares how sensitive traditional management techniques are to detecting nitrate-nitrogen effects on the benthic invertebrate community. Chapter Three builds on Chapter Two by investigating non-traditional multivariate ordination techniques. In this chapter I aim to address potential limitations in traditional biotic metrics by measuring different ecological community aspects across a nitrate-nitrogen gradient. Chapter Four integrates the results from Chapters Two and Three and discusses the wider implications of the results for freshwater management.

Chapter 2: Effects of nitrate-nitrogen on benthic invertebrate biotic metrics

Introduction

Aquatic ecosystems globally are under stress from excessive inputs of nitrate-nitrogen and phosphorus (Carpenter et al. 1998). In freshwater ecosystems excessive nitrate-nitrogen can cause algal blooms and anoxia leading to alterations in aquatic community structure and ecosystem functions (Vitousek et al. 1997; Carpenter et al. 1998). Coastal streams in the Canterbury Plains are no exception to excessive nutrients, with high nitrate-nitrogen concentrations (e.g. > 2mg/L) recorded in surface waters (Stevenson et al. 2010). Despite these high levels of nitrate-nitrogen surprisingly little research has been done in New Zealand on the response of stream communities to high levels. Hickey (2013) has proposed four nitrate-nitrogen categories (or bands) that might reflect differing levels of effects on benthic macroinvertebrates and fish. These bands were based primarily on literature reviews and limited ecotoxological trials. Currently, considerable attention has been given to these categories, which will be embedded in the National Policy Statement for Freshwater Management and National Objectives Framework (Ministry for the Environment 2013). These nitrate-nitrogen concentration bands rate community protection based on conservation value of the ecosystem, and then attribute a chronic (annual median) and acute (annual 95th percentile) guideline value (Hickey 2013). For example, the lowest classification proposed is 80% protection, with an acute value of 20 mg/L and chronic value of 6.9 mg/L (Hickey 2013). However, these values are based primarily on overseas ecotoxological trails with few representative species from New Zealand. Therefore, there is a need

to supplement them with empirical evidence based on New Zealand stream fauna. My study provides field evidence to determine the proposed bands applicability to Canterbury Plain streams.

Benthic invertebrates are small aquatic animals that inhabit stream and river substrates including sediments, macrophytes, debris, logs and filamentous algae (Rosenberg & Resh 1993). Benthic invertebrates are widely used as indicators of pollution and stream health (Stark 1998). Therefore they might be expected to provide useful biotic indicators of nitrate-nitrogen environmental stress in Canterbury streams. The advantages of using benthic invertebrates include their ubiquitous nature in streams and rivers, their diverse taxonomy and occupation of different aquatic niches essential to the food web (Rosenberg & Resh 1993). They also provide important linkages in food webs between primary consumers and higher predators (Winterbourn 2004b), and provide ecosystem services including processing of organic matter, removal of suspended particulates and modifiers of aquatic habitats (Winterbourn 2004a). Alteration to food web links, for example between invertebrate grazers and consumption of primary productivity, may indicate a stress or subsidy effect by nitrate-nitrogen (Niyogi et al. 2007b). As some benthic invertebrates are sedentary in nature and have an annual life cycle (Collier & Winterbourn 2000) they are also useful for the longer-term monitoring of environmental stressors (Rosenberg & Resh 1993). Furthermore, benthic invertebrates are relatively easy to identify and we understand the roles of different invertebrate species in the freshwater ecosystem (Winterbourn, Gregson & Dolphin 2000). These benefits of benthic invertebrates as biological indicators of environmental stress underpin their wide use for monitoring 'stream health' in New Zealand (Quinn & Hickey 1990; Rosenberg & Resh 1993; Hall et al. 2001; Winterbourn 2004b).

The tolerances of some benthic taxa to organic pollution has been suggested in the Taranaki Ring Plain in New Zealand (Stark 1985; Stark 1998). However, these tolerances are based on subjective opinion rather than empirical testing. Stark (1985) allocated different tolerances of taxa based on to a 0 – 10 scale, where higher values indicated increasing sensitivity to organic pollution. Ephemeroptera, Plecoptera and Trichoptera generally have high values (e.g. ~ 8) while tolerant taxa including Annelida and Mollusca have

low values (e.g. ~ 3) (Stark 1985; Stark 1998). These taxa values were then used to calculate site scores termed the “Macroinvertebrate Community Index” (MCI). The MCI summarizes the invertebrate community state reflecting water quality and impact of organic pollution. For example, in a stream with high organic pollution, sensitive invertebrate taxa should decline in density or be excluded, where as tolerant taxa should increase in density and become dominant in the invertebrate community (Stark & Maxted 2007). The MCI, along with the semi-quantitative (SQMCI) and quantitative variants (QMCI), is used widely throughout New Zealand (Stark 1985; Stark 1998; Stark & Ngaire 2009; Wright-Stow & Winterbourn 2010). Although originally intended to provide a single number to communicate a measure of stream health to managers, the simplicity of the MCI has allowed scientists to characterize community complexity and has been generally shown to work well in New Zealand (Stark & Maxted 2007). Investigating the tolerances and comparing MCI tolerance scores of New Zealand benthic invertebrates between Taranaki and Canterbury is important to understand if regional differences in organic pollution exist. Also to understand if the MCI score is sensitive enough to detect community shifts within a degraded region (Stark & Maxted 2007). These points are crucial for management to understand if the MCI is to be used as a method to assess stream health in Canterbury streams.

The current New Zealand management toolbox for evaluating ‘stream health’ using benthic invertebrates is based primarily on structural biotic metrics of community composition (Rosenberg & Resh 1993; Meredith et al. 2000; Gray 2013). These metrics summarize complex biological data into single numbers for communication with environmental management and the general public (Stark 1998). Biotic metrics often focus on a particular aspect of community structure (e.g. dominance of certain taxa) that might then be expected to shift across a gradient of water quality. Common biotic metrics calculated from invertebrate communities include species richness, abundance, sensitive taxa richness, dominance of insensitive taxa (e.g. % Mollusca), number of different feeding modes, MCI and community evenness (Winterbourn 2004b).

Stable isotope analysis (particularly using carbon and nitrogen) has been widely used to discriminate energy sources and determine trophic structure in freshwater ecosystems (Peterson & Fry 1987). For example, the diet-tissue fractionation ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) for benthic macroinvertebrate grazers (e.g. *Deleatidium*) can provide insights into food quality (Webb, Hedges & Simpson 1998). Diet quality can be measured as the diet-tissue ratio of carbon to nitrogen, where lower values indicate higher food quality (Liess et al. 2012). Differences in food quality are assumed to relate to nitrogen (N) content of algae consumed (Jardine et al. 2005). Therefore, if nitrate-nitrogen concentrations alter the N content and food quality of algae, fractionation ratios of carbon and nitrogen should change in the diet-tissue of benthic invertebrate grazers (Rounick, Winterbourn & Lyon 1982; Peterson & Fry 1987). This technique may provide a mechanistic explanation of how nitrate-nitrogen alters benthic invertebrate community composition through changes in dietary resources.

Aims of this chapter

The aim of this chapter was to investigate the effects of nitrate-nitrogen on benthic invertebrates using common biotic metrics. Thus I will test how useful biotic metrics are as ecological indicators of nitrate-nitrogen. Understanding if nitrate-nitrogen alters food quality will also be studied to investigate possible mechanistic pathways. Together these aims will provide information to advise proposed nitrate-nitrogen bands and appropriate management techniques for identifying nitrate-nitrogen effects.

Methods

Location and selection of survey streams

I conducted a field survey of 41 lowland streams in the Canterbury Plains, South Island, New Zealand. Sites were located primarily around Ashburton, Lincoln, and Rangiora spanning a gradient of nitrate-nitrogen (Figure 2). Sites were selected from a preliminary field survey conducted during November and December 2012. This preliminary survey identified 100 sites where physical and water chemistry properties were measured. In order to reduce confounding physico-chemical effects I selected a sub-set with generally similar stream wetted width (1-2m), substrate size (~5 on the Wentworth scale substrate index), temperature (15-20°C), pH (6-8) and dissolved oxygen (>70%). At the 41 streams I sampled physical and chemical properties and benthic macroinvertebrates on a single occasion at each site between February and March 2013 under baseflow conditions. Grab water samples were collected on six occasions at each site, once per month until August 2013. I was therefore able to measure the temporal variation in nitrate-nitrogen concentrations from summer to winter at all 41 sites.

Field methods

Each site was a 20m reach, including at least one riffle – run complex. A range of physical variables was measured at each site. Stream stability and riparian characteristics were measured using the channel stability score of Pfankuch (1975) and procedure P2d in (Harding et al. 2009). Stream discharge was estimated from three mean velocity measurements (measured with a March-McBirney Flo-Mate 2000), wetted width and mean of three stream depths across a stream transect. Stream shading was estimated from a single densiometer reading taken at the middle of the stream reach (Model-A, Robert E. Lemmon, Forest Densiometer). Stream substrate size was visually estimated over the reach using the Wentworth scale by estimating percentage of each size class present (Wentworth 1922). Spot water chemistry parameters were measured in the field using meters that measured pH, specific conductivity (YSI

Incorporated, model 63-10F7), dissolved oxygen, temperature (YSI Incorporated, model 550A) and turbidity (HACH Turbidimeter, model 2100P). On each sampling occasion grab water samples (125ml) were taken and filtered on site using a 30ml syringe (BD Plastipak) with glass fiber filter paper (0.45 μm, Labserv) and transported in ice and frozen in the laboratory before processing for nitrate-nitrogen.

Benthic invertebrate densities were quantitatively sampled from three Surber samples (0.25m^2, 0.5mm mesh), which were taken in riffles. Benthic invertebrate diversity was calculated by combining data from a single composite kick net (0.5mm mesh) sample taken in riffle and bank habitats and the three Surbers. Samples were preserved in 70% ethanol in the field. In the laboratory, invertebrate samples were washed through a 500µm sieve, sorted and identified to genus or lowest taxonomic group possible using standard keys (Winterbourn et al. 2000). Stable isotope analysis was conducted on the gut contents of the common invertebrate grazer, *Deleatidium.* They were collected in field sites using a kick net and transported in a chill box before being frozen and processed at a later date for δ 15N and δ 13C stable isotopes. Isotope samples were prepared by removing the guts and drying them to produce 1mg ± 0.15mg. The sample was ground, wrapped in tin and transported to the UC Davis Stable Isotope Facility for processing of the fractionation ratio of δ $C1^3/C^{12}$ and δ N^{15}/N^{14} stable isotope natural abundance signatures. Only 26 sites contained *Deleatidium* with the required 1mg of dry biomass gut contents for stable isotope analysis.

Laboratory methods

Nitrate-nitrogen concentrations (mg/L) in water were analysed using two methods. The primary method, conducted on Easychem plus machine (Analytical technologies – SYSTEA), used cadmium to reduce nitrate-nitrogen to nitrite-nitrogen for colorimetric measurement to infer the nitrate-nitrogen concentration (Systea Scientific 2006). A secondary confirmation method using a UV spectrophotometer (UV-1800 – Shimadza) was used to test diluted samples that exceed the standard curve of the primary method. Alkalinity was estimated from acid-base titrations in the laboratory (Eaton et al. 2005).

Regional and seasonal nitrate-nitrogen trends

Nitrate-nitrogen concentrations were tested for variation in time and between sub-regions of Ashburton, Rangiora and Lincoln. A two-factor ANOVA analyzing: region, month and region*month interaction was conducted to test the spatial and temporal variation in six nitrate-nitrogen spot samples. A linear or non-linear regression model was selected based on Akaike Information Criterion (AIC) scores.

Selection of a nitrate-nitrogen variable

Three nitrate-nitrogen variables were used to summarize the six-monthly nitrate-nitrogen data; mean, median and maximum nitrate-nitrogen concentrations. Linear regression was used to test the relationship between these nitrate-nitrogen variables to identify a single suitable nitrate-nitrogen variable for comparison with benthic macroinvertebrate biotic indices.

To determine how typical my six monthly nitrate-nitrogen samples were of long-term nitrate-nitrogen regimes at my sites a comparison between long-term data collected before the survey and my data was conducted. Long-term data was collected from Environment Canterbury from the year prior to my survey (February 2012 – February 2013). I was able to do this for 15 field survey sites where Environment Canterbury data was available. Linear regression was used to test the relationship between the mean nitrate-nitrogen concentration of the long-term data and my data.

Detection of potentially confounding environmental variables

Confounding variables were identified for further analysis. Other environmental variables may exhibit gradients across the 41 field survey sites that are not related to mean nitrate-nitrogen, however may also affect benthic invertebrate composition. Environmental variables significantly correlated with the mean nitrate-nitrogen gradient were first identified using a correlation table of the 11 environmental variables measured in the field with R statistical software package (v3.02). The environmental variables significantly correlated with mean nitrate-nitrogen were then removed due to auto-correlation. The remaining

environmental variables were analyzed using non-metric multidimensional scaling (NMDS) in the R community ecology package Vegan (Oksanen 2011). This technique identified significant environmental gradients across the 41 field survey sites not correlated to mean nitrate-nitrogen. The NMDS ordinates environmental data based on the similarity of environmental variables at each site (as similarity conducted by Scarsbrook 2002). Fitted significant environmental gradients on the ordination indicate environmental variables are significantly different between field sites; tested using Monte Carlo 999 permutation tests (Oksanen 2011). NMDS goodness of fit is measured as stress on a scale between 0 – 1 with values close to 0 representing a better model fit to the data.

Confounding variables were tested with multiple linear regression using adjusted sums of squares (Type 3) approach (Zar 2010). The simplest multiple linear regressions were selected by removing non-significant environmental variables from the model before testing the effect of mean nitrate-nitrogen.

Biotic metrics and stable isotopic analysis

Twelve biotic metrics were tested for a linear relationship with mean nitrate-nitrogen. These included; total taxonomic richness which was measured to represent the number of benthic invertebrate niches. Functional feeding group richness which includes the number of different feeding modes (e.g. shredders, collector-browsers and predators) (Cummins & Klug 1979). The ratio of Ephemeroptera, Plecoptera and Trichoptera (EPT) richness and abundance to total richness and abundance respectively provides an indicator for pollution sensitive taxa (Stark 1985; Stark 1998). The Shannon-Weaver and Simpson diversity indices are comparative indicators of community evenness that measure how similar in number species communities are in the environment (Shannon 1948; Simpson 1949). Berger-Parker Dominance index measures the numerical importance of the most abundant species (Berger & Parker 1970). Environment Canterbury measure invertebrate health based on biotic metrics of ratios of certain taxonomic groups (Meredith et al. 2000). %EPT2 represents the proportion of taxa belonging to EPT groups except the pollution tolerant

caddisflies *Oxyethira* and *Paroxyethira*. %EPT3 / (% Oligochaeta + % Chironomidae) indicates the ratio of percent EPT3 (EPT taxa excluding pollution tolerant *Oxyethira*, *Paroxyethira* and *Aoteapsyche*) to ratio of percent Oligochaeta plus percent Chironomidae. %EPT3 / (% Mollusca + % Crustacea) represents the ratio of percent EPT3 to percent Mollusca plus percent Crustacea.

The relationships between the fractionation ratio of $\delta\ C1^{3}/C^{12}$ and $\delta\ N^{15}/N^{14}$ stable isotope natural abundance signatures and mean nitrate-nitrogen were compared using linear regression. Only 26 sites contained isotopic signatures from the mayfly grazer *Deleatidium*.

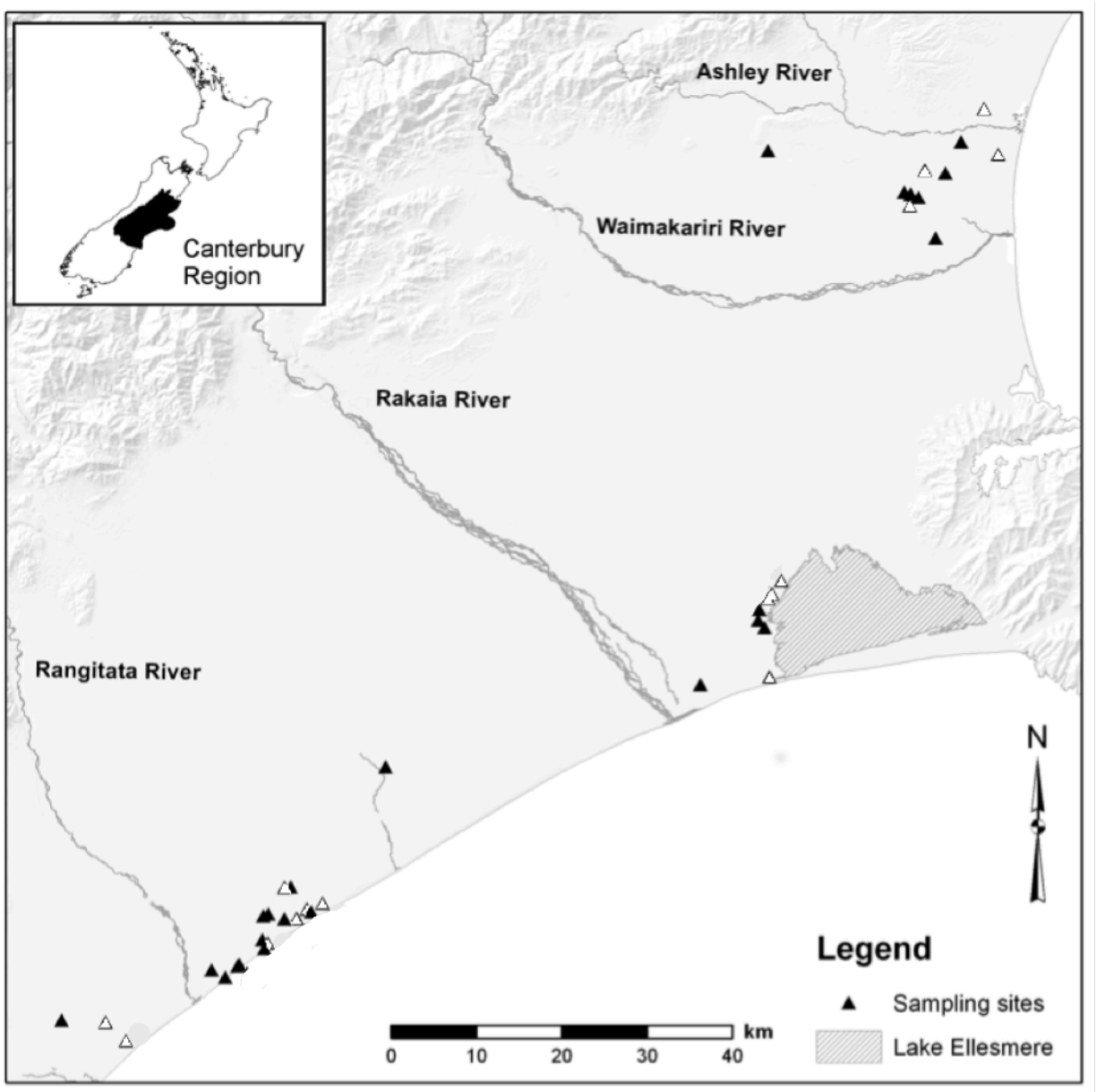

Figure 2: Location of 41 sites used for the field survey on the Canterbury Plains, South Island, New Zealand. Open triangles represent the 15 sites with long-term Environment Canterbury water quality data.

Results

Regional and seasonal nitrate-nitrogen trends

Nitrate-nitrogen concentrations were expected to vary regionally and over time across the 41 streams. Spot samples of nitrate-nitrogen were significantly different between the three sub-regions over the six months from March to August 2013 (Figure 3, Table 1). However, no interaction was detected between sub-region and month (Table 1).

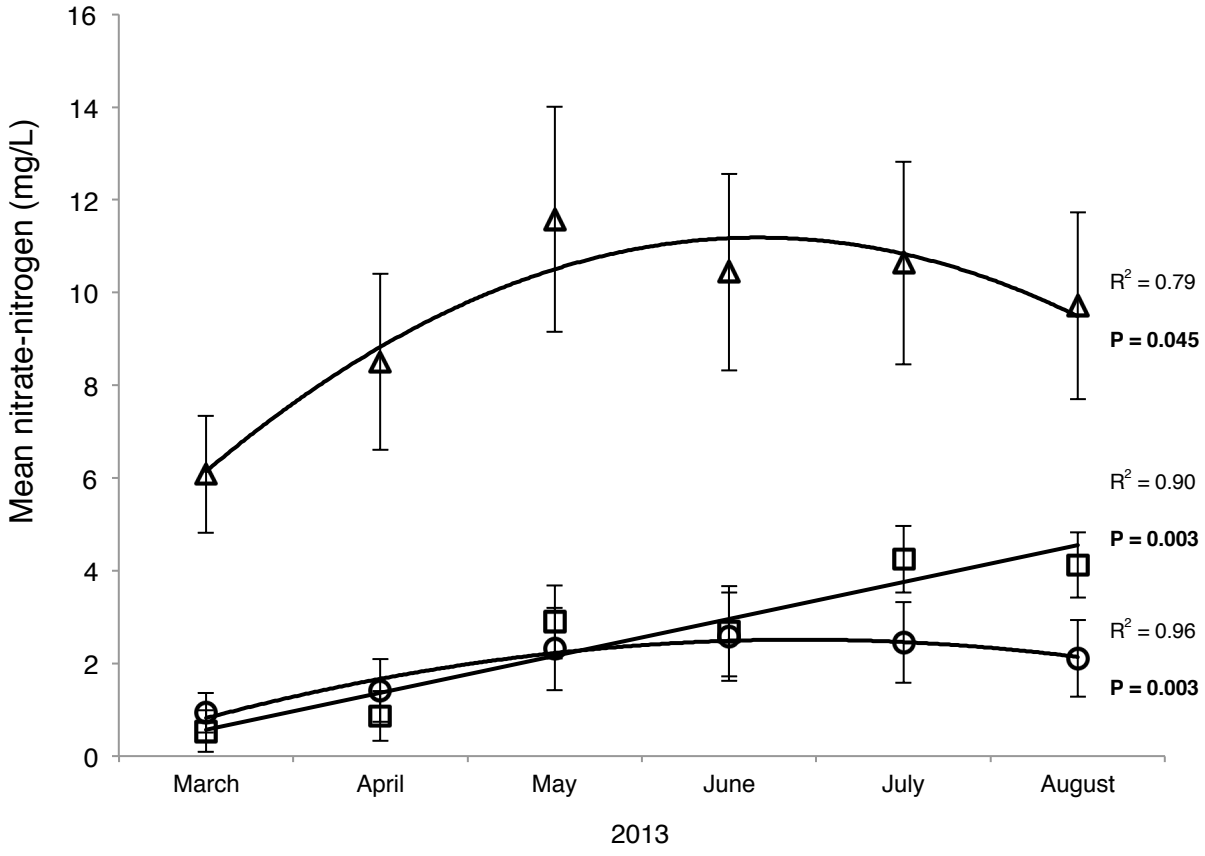

Figure 3: Comparison of mean nitrate-nitrogen (mg/L) in 41 streams over six months between March and August 2013 from three sub-regions of Ashburton (triangles), Lincoln (squares) and Rangiora (circles). Error bars indicated standard error of the mean.

Nitrate-nitrogen concentrations for Ashburton streams markedly exceeded those measured in Rangiora and Lincoln (Figure 3). Unimodel relationships provided the best fit of nitrate-nitrogen trends based on AIC scores for Ashburton (R^2 = 0.79, $F_{2,3}$ = 10.37, P < 0.045) and Rangiora (R^2 = 0.98, $F_{2,3}$ = 64.43, P < 0.003)(Figure 3). A linear relationship was the best fit for the Lincoln nitrate-nitrogen trend (R^2 = 0.90, $F_{2,3}$ = 45.11, P < 0.003) (Figure 3).

Table 1: Two-factor ANOVA results displaying the effect of sub-region, month and interaction on nitrate-nitrogen concentrations collected from 245 spot samples. Significance is at P < 0.05 indicated in bold

Variable	DF	Sum SQ	Mean SQ	F Value	P Value
Region	2	2185.18	1092.59	150.60	**< 0.001**
Month	5	329.74	65.95	9.90	**< 0.001**
Region*Month	10	88.80	8.88	1.22	0.2765

Selection of a nitrate-nitrogen variable

A positive relationship was found between mean and maximum nitrate-nitrogen (R^2 = 0.997, F_{40} = 2722.47, P < 0.001) and mean and median nitrate-nitrogen (R^2 = 0.996, F_{40} = 4517.93, P < 0.001) (Figure 4). Since nitrate-nitrogen variables of median and maximum were highly related to mean nitrate-nitrogen this was selected as the nitrate-nitrogen variable for comparison with benthic invertebrate biotic metrics.

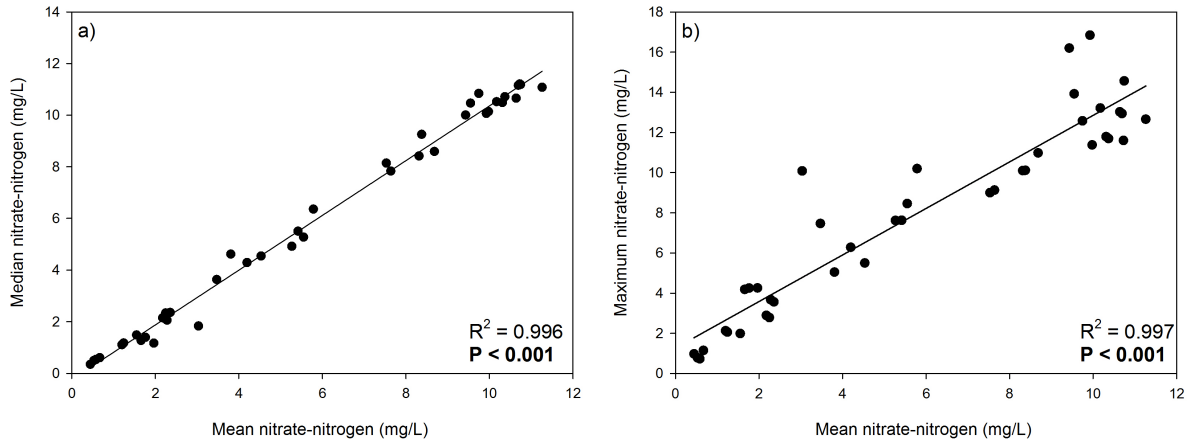

Figure 4: Relationship between; a) mean and median and; b) mean and maximum nitrate-nitrogen (mg/L) for 41 field survey sites.

The Long-term mean nitrate-nitrogen (Environment Canterbury 2012-2013) and my data were positively correlated (R^2 = 0.94, F_{14} = 192.8, P < 0.001) (Figure 5).

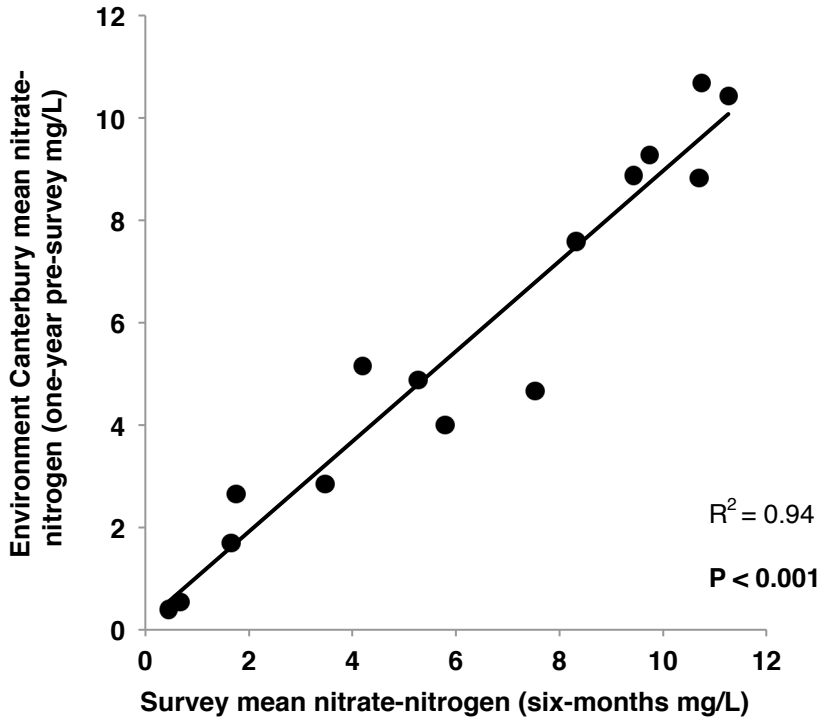

Figure 5: Relationship between mean nitrate-nitrogen (mg/L) from Environment Canterbury data collected between January 2012 to January 2013 and my six months of nitrate-nitrogen data collected from March 2013 to August 2013 at the same 15 sites.

Detection of potentially confounding environmental variables

I tested for correlations between environmental variables to identify spatially auto-correlated variables with mean nitrate-nitrogen. Conductivity and percent dissolved oxygen were positively correlated and turbidity was negatively correlated with mean nitrate-nitrogen (Table 2). As these environmental variables were correlated with mean nitrate-nitrogen they were removed from the analysis to test for other environmental gradients. An NMDS ordination of environmental variables showed discharge (R^2 = 0.86, P < 0.001) and shading (R^2 = 0.92, P < 0.001) provided significant environmental gradients (999 permutations) across my sites (Figure 6).

Table 2: Correlation between 11 environmental variables from 41 field survey sites collected in March 2013. Correlation variables scaled from -1 to 1 and displayed at two decimal places. * Indicates p < 0.05.

	Nitrate	Alkalinity	Conductivity	Densiometer	Shading	DO %	Pfankuch	pH	Channel Stability evaluation	Temperature
Alkalinity	0.06									
Conductivity	**0.54*** p > 0.001	0.16								
Shading	0.00	-0.14	-0.09							
Discharge	0.13	0.31	**-0.34*** p = 0.028	0.09						
DO %	**0.35*** p =0.012	0.29	0.01	-0.07	**0.38*** p = 0.022					
Pfankuch	-0.07	-0.04	-0.15	-0.02	0.02	-0.04				
pH	-0.04	-0.15	0.04	-0.24	-0.22	**0.35*** p = 0.006	-0.06			
Channel stability evaluation	0.22	0.11	-0.15	0.00	0.17	0.01	0.00	-0.11		
Temperature	-0.22	-0.12	0.04	-0.16	-0.17	0.00	-0.03	0.30	-0.28	
Turbidity	**-0.31*** p = 0.048	-0.25	-0.28	-0.09	0.01	0.01	0.28	**0.42*** p = 0.006	0.04	0.26

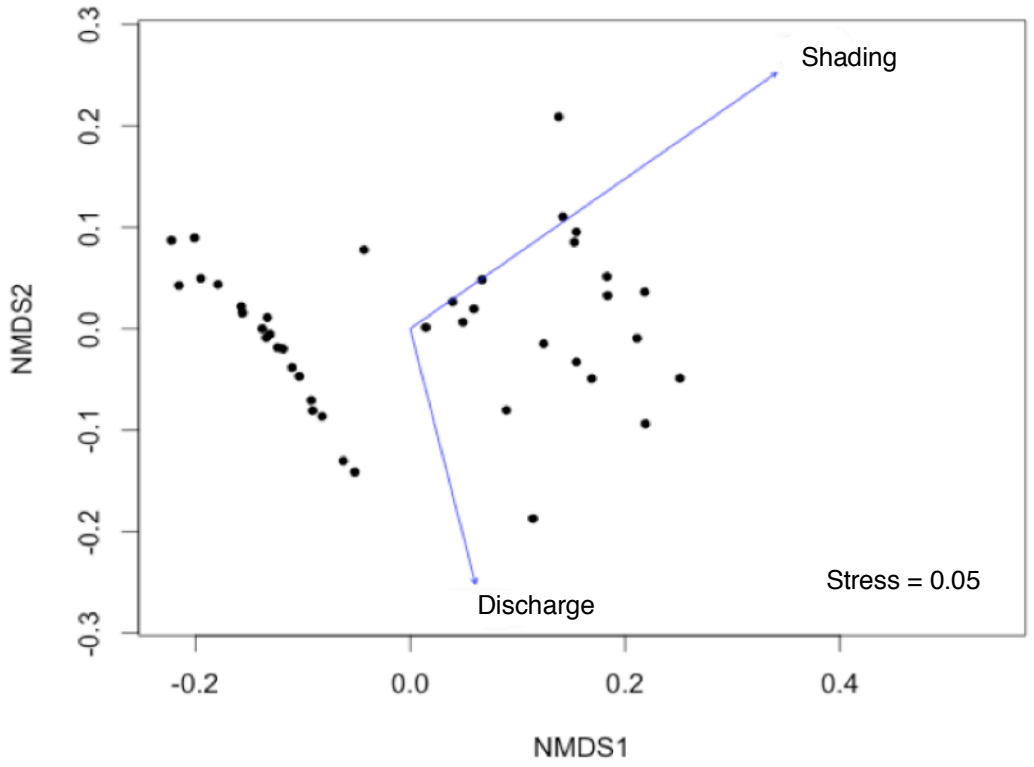

Figure 6: Ordination of 7 environmental variables (alkalinity, shading, discharge, channel stability evaluation score, pH, substrate index and temperature) not correlated to the nitrate-nitrogen environmental gradient. Significant gradients displayed on ordination plot.

Relationships between biotic metrics and nitrate-nitrogen

Comparisons of mean nitrate-nitrogen against 12 common invertebrate biotic metrics showed no statistically significant relationships, even after accounting for confounding variables (Figure 7, Tables 3, 4). The MCI scores for the 41 sites ranged between 80-100 representing the probable moderate pollution classification (Figure 7). In contrast, QMCI scores were variable and sites with similar nitrate levels had QMCI scores that spanned poor to excellent pollution categories proposed by Stark (1998)(Figure 7). Of the other environmental variables, discharge was positively related to the biotic metrics of percent EPT abundance, %EPT2 and %EPT3 / (% Mollusca + % Crustacea) and negatively related to functional feeding group richness (Table 3).

Table 3: Multiple regression results between mean nitrate-nitrogen and discharge with biotic metrics.

Dependent variable	Independent variable	Estimated regression coefficients	P-Value	Overall R^2
Functional feeding group richness	Mean nitrate-nitrogen	+ 0.14	0.63	0.26
	Discharge	- 3.86	< 0.001	
Percent EPT abundance	Mean nitrate-nitrogen	- 0.00	0.68	0.23
	Discharge	+ 0.32	< 0.001	
%EPT2	Mean nitrate-nitrogen	- 0.00	0.68	0.18
	Discharge	+ 0.27	0.003	
%EPT3 / (% Mollusca + % Crustacea)	Mean nitrate-nitrogen	- 0.01	0.79	0.19
	Discharge	+ 2.17	0.005	

Table 4: Linear regression results for the relationship of mean nitrate-nitrogen with biotic metrics.

Dependent variable	Independent variable	F-Value	P-Value	R^2
Taxa richness	Mean nitrate-nitrogen	$F_{1,39} = 0.01$	0.93	0.00
Functional feeding group richness	Mean nitrate-nitrogen	$F_{1,39} = 0.29$	0.60	0.01
Percent EPT richness	Mean nitrate-nitrogen	$F_{1,39} = 0.01$	0.93	0.00
Percent EPT abundance	Mean nitrate-nitrogen	$F_{1,39} = 0.23$	0.63	0.01
MCI	Mean nitrate-nitrogen	$F_{1,39} = 0.41$	0.53	0.01
QMCI	Mean nitrate-nitrogen	$F_{1,39} = 0.41$	0.62	0.00
Shannon diversity	Mean nitrate-nitrogen	$F_{1,39} = 0.21$	0.65	0.01
Simpson diversity	Mean nitrate-nitrogen	$F_{1,39} = 0.22$	0.64	0.01
Berger-Parker dominance	Mean nitrate-nitrogen	$F_{1,39} = 0.63$	0.43	0.02
%EPT2	Mean nitrate-nitrogen	$F_{1,39} = 0.22$	0.64	0.01
%EPT3 / (% Oligochaeta + % Chironomidae)	Mean nitrate-nitrogen	$F_{1,39} = 1.01$	0.32	0.00
%EPT3 / (% Mollusca + % Crustacea)	Mean nitrate-nitrogen	$F_{1,39} = 0.11$	0.74	0.00

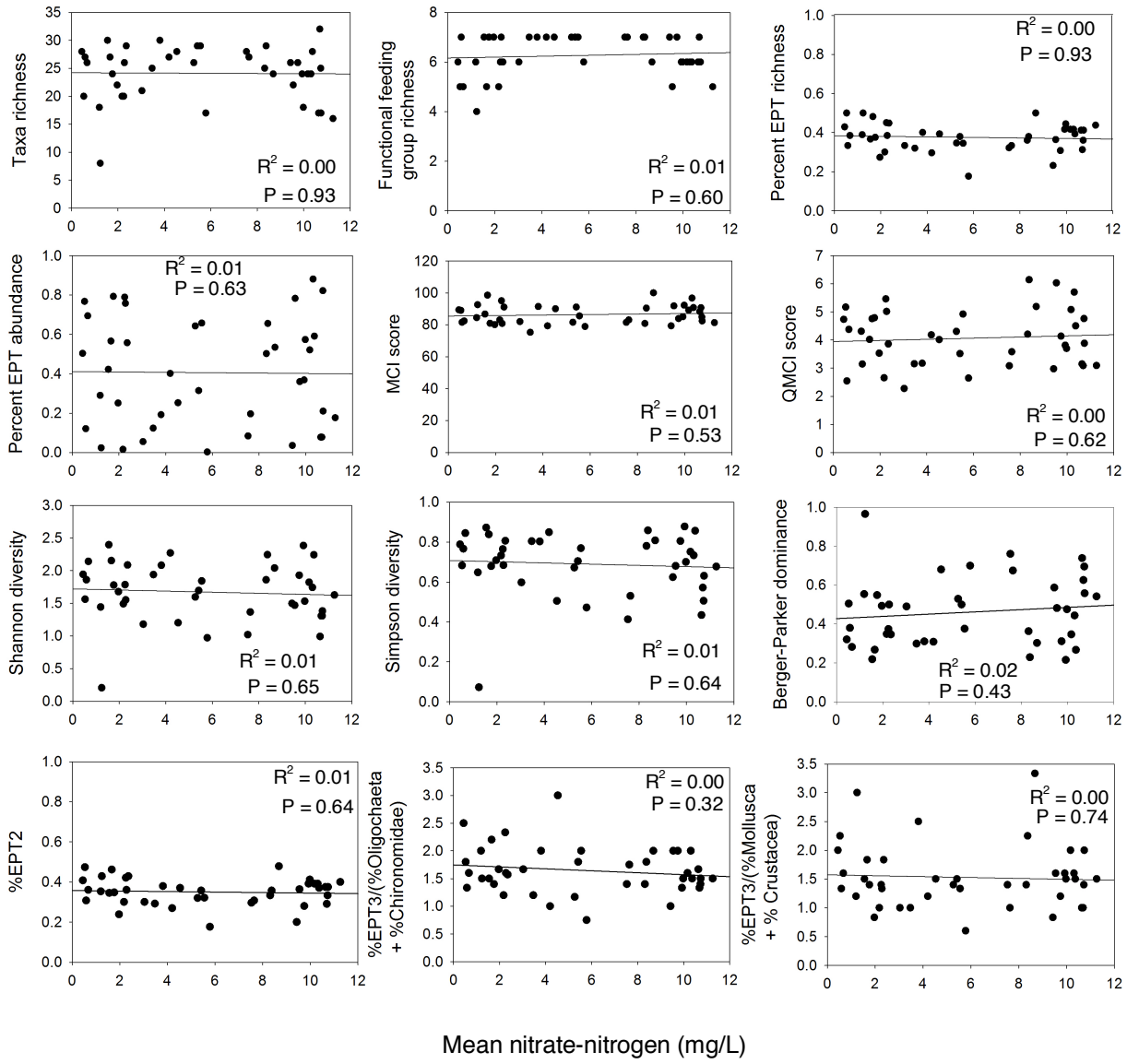

Figure 7: Relationships between mean nitrate-nitrogen (mg/L) and biotic metrics of; taxa richness, functional feeding group richness, percent EPT richness, percent EPT abundance, MCI score, QMCI score, Shannon diversity, Simpson diversity, Berger-Parker dominance, %EPT2 taxa, the ratio of %EPT3 to % Oligochaeta + % Chironomidae and the ratio of %EPT3 to % Mollusca + % Crustacea from 41 sites.

Relationship between stable isotopes of grazer gut contents and nitrate-nitrogen

A significant but weak negative linear relationship occurred between mean nitrate-nitrogen and the ratio of δ $C1^3/C^{12}$ and δ N^{15}/N^{14} (R^2 = 0.21, F_{25} = 7.82, P < 0.001) and δ N^{15}/N^{14} (R^2 = 0.15, F_{25} = 5.26, P < 0.03) (Figure 8). However, no significant relationship was determined between δ $C1^3/C^{12}$ and mean nitrate-nitrogen (R^2 = 0.04, F_{25} = 1.91, P < 0.18) (Figure 8).

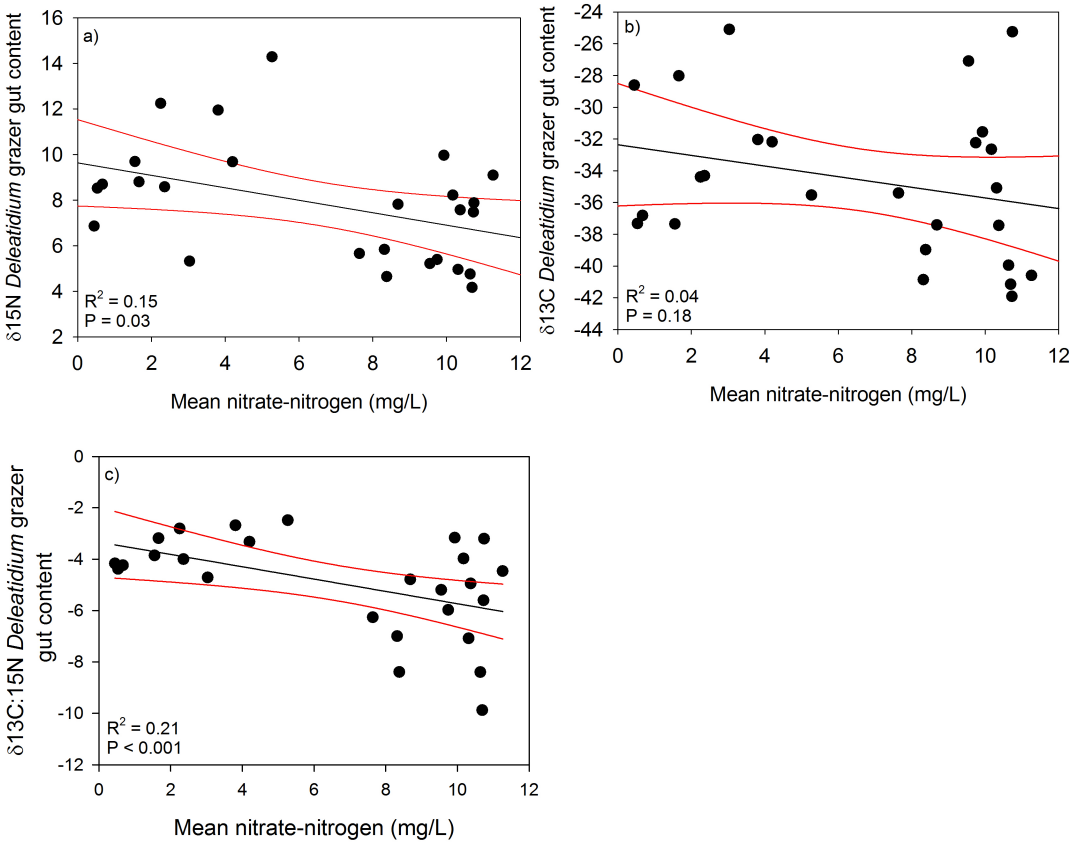

Figure 8 : Relationships between mean nitrate-nitrogen and; a) *δ N^{15}/N^{14}*; (b) *δ $C1^3/C^{12}$* and c) ratio of *δ $C1^3/C^{12}$* and δ N^{15}/N^{14} of *Deleatidium* gut tissue collected during the field survey in 26 sites. Red lines indicate 95% confidence intervals.

Discussion

Seasonal and regional nitrate-nitrogen concentrations

Nitrate-nitrogen concentrations varied between season and sub-region over the six months (March to August 2013) after field survey sampling for Ashburton, Rangiora and Lincoln. This seasonal variation showed similar patterns in mean, median and maximum concentrations. As my study aimed to determine the response of invertebrate communities to nitrate-nitrogen I needed to be confident that my spot water chemistry data provided a true representation of long-term water chemistry at the sites that I collected benthic invertebrates. This comparison of Environment Canterbury's 12 monthly data with my six monthly spot data showed the same patterns and range of values. Therefore, benthic invertebrate communities that I sampled during my survey were highly likely to be exposed to similar nitrate-nitrogen concentrations as I recorded. In all sub-regions mean nitrate-nitrogen concentrations increased from March through the winter, however Ashburton and Rangiora peaked during May, whilst Lincoln continued to rise through to August. This indicates the highest nitrate-nitrogen concentrations for Lincoln may not have been measured, and frequent monitoring is required to estimate the nitrate-nitrogen regime in a stream. Consequently, estimation of nitrate-nitrogen regimes for management purposes requires frequently sampling, especially during winter when nitrate-nitrogen concentrations increase.

The Ashburton sub-region had markedly higher mean nitrate-nitrogen and a more pronounced unimodel relationship than Rangiora and Lincoln. As particularly high nitrate-nitrogen has been observed in Ashburton groundwater (Wong & Hanson 2012) and spring-fed surface waters (Stevenson et al. 2010) this is not surprising. Seasonal fluctuations of nitrate-nitrogen observed in field survey streams were similar to patterns recorded in Canterbury groundwater, where higher concentrations were observed in winter and lower concentrations during summer (Hanson 2002). These trends may be explained by seasonal changes in soil moisture content; winter has increased rainfall recharge, lower evaporation rates and reduced plant activity, which facilitates transportation of nitrate-nitrogen into groundwater. In contrast, summer moisture content is

lower due to less available water, and nitrate-nitrogen is retained within the agricultural landscape (Hanson 2002). Additionally, this seasonal pattern could be in part due to a drought during the summer of 2013 (Statistics New Zealand 2013), which could have retained high nitrate-nitrogen in the soils and subsequently released during heavy rainfall in April/May (NIWA National Climate Center 2013). Therefore, Ashburton may have had higher nitrate-nitrogen retained in the soil, which may explain the increase in nitrate-nitrogen concentrations and greater seasonal variation than Rangiora and Lincoln streams.

Environmental variables and biotic metrics

Although my study aimed to reduce confounding variables, shade and discharge exhibited detectable gradients across my sites, with discharge positively related to percent EPT abundance, %EPT2 and %EPT3 / (% Mollusca + % Crustacea), and negatively related to functional feeding group richness. Similarly, Greenwood et al. (2012) found discharge was positively related to invertebrate gradients, particularly percent EPT and QMCI scores in Canterbury streams. Low flows have been shown to alter macroinvertebrate abundance and richness, and may act as a greater stressor when coupled with high nutrients (Suren et al. 2003; Matthaei et al. 2010). The response of invertebrate communities to flow reduction can depend on water quality, with invertebrate communities at more pristine sites more likely to respond than degraded sites (Death, Dewson & James 2009). Interestingly, functional feeding group richness was negatively related to increased discharge, and may be a result of stream channelisation; an increase in flow decreases slow-flowing water that removes feeding niches (Quinn et al. 1992). Overall, since invertebrate communities respond to discharge, this indicates taxa may not have broad ecological tolerances to reduced flow, and thus discharge is an important environmental variable in the Canterbury Plains.

Nitrate-nitrogen and biotic metrics

An important result of this analysis is that traditional stream health metrics were not related to mean nitrate-nitrogen in my streams, even after removing variation associated with discharge and shading. Large ranges

in biotic metric values spanning the mean nitrate-nitrogen gradient (0.4 – 11.3 mg/L) indicated biotic metrics had no sensitivity to nitrate-nitrogen. Although no relationship was detected this does not necessarily mean nitrate-nitrogen is not influencing benthic invertebrate communities in Canterbury. Several nationwide studies have related structural metrics and shifts in ecosystem functions at much lower values of ~ 0.3 – 3.2 mg/L in national-wide studies (Clapcott et al. 2010; Clapcott et al. 2012). Similarly, subsidy-stress relationships between invertebrate communities and nitrate-nitrogen were observed across a gradient of 0 – 1.8mg/L in Otago streams (Riley et al. 2003; Niyogi et al. 2007b). Furthermore, declines in MCI scores in Manawatu streams were observed from 0.01 – 1.9 mg/L of nitrate-nitrogen (Death). In each of these studies the range where effects of nitrate-nitrogen were observed are much lower than the gradient in my Canterbury Plains streams. Therefore, the concentrations in which nitrate-nitrogen effects communities could be lower than occurs in most of my survey sites. Locating and sampling very low nitrate-nitrogen sites in the Canterbury Plains could provide this range, and would be a useful focus for future studies.

Although MCI and QMCI indices were developed to evaluate organic pollution, scores were variable and unrelated to mean nitrate-nitrogen in the Canterbury Plains. The QMCI classified sites from poor to excellent whilst MCI scores only represented the probably moderate pollution class. This inconsistency between metrics indicates the MCI and QMCI would give conflicting results in some Canterbury Plains waterways and that they would be of little use in detect possible nitrate-nitrogen effects. An explanation for why the MCI and QMCI perform well to identify changes in community composition related to land-use and not nitrate-nitrogen in the Canterbury Plains may be an issue of spatial scale. Changes in benthic community composition between land-uses result in a larger shift in composition than seen in the Plains, where most streams are already degraded to some degree. The MCI and QMCI scores may not be sensitive to these subtle effects. Overall, the use of MCI and QMCI indices cannot be advised to detect shifts in the already pollution tolerant streams of the Canterbury Plains.

Nitrate-nitrogen and stable isotopes of grazer gut contents

Gut C:N ($\delta C1^3/C^{12}$ to $\delta N^{15}/N^{14}$) ratio and N ($\delta N^{15}/N^{14}$) of a common invertebrate grazer, *Deleatidium,* declined with increasing mean nitrate-nitrogen concentrations. This indicates that food quality may be increasing with mean nitrate-nitrogen, as lower values are considered to represent higher food quality (Liess et al. 2012). This is not surprising given nitrate-nitrogen is a limiting resource for algae, and higher availability results in higher up-take by the algal community (Jardine et al. 2005). An Otago study found algae C:N ratios were more strongly related to nitrogen availability than phosphorus, and predicted algal taxa richness and taxon richness better than dissolved nutrient values (Liess et al 2012). Although algal C:N ratios were not measured in this study, the change in gut content C:N ratios could thus provide a mechanism for which mean nitrate-nitrogen could affect invertebrate taxa composition. Furthermore, Clapcott et al (2012) found N in primary consumers, along with nitrate-nitrogen + nitrite-nitrogen, were strong indicators of land-use activities. Therefore, investigations into how mean nitrate-nitrogen changes resources available for consumption by benthic invertebrates may provide important information of potential mechanisms nitrate-nitrogen could affect freshwater ecosystems.

Conclusion

Nitrate-nitrogen undergoes seasonal and regional fluctuations that require frequent sampling to better estimate regimes in the Canterbury Plains. In comparison to Rangiora and Lincoln, the Ashburton region had the highest nitrate-nitrogen concentrations. Mean nitrate-nitrogen was not related to biotic metrics, possibly due to the semi-degraded condition of invertebrate communities in these streams. A negative relationship with N and C:N ratio of a common grazers gut contents was determined, potentially indicating a mechanic pathway of nitrate-nitrogen to influence invertebrate taxa composition. Overall, biotic metrics were of limited use in detecting any possible nitrate-nitrogen related effects on community composition.

Chapter 3: Patterns between gradients of invertebrate composition and nitrate-nitrogen

Introduction

The lack of response of benthic invertebrate biotic metrics to nitrate-nitrogen described in Chapter 2 indicates that either nitrate-nitrogen does not affect invertebrate composition, or that biotic metrics have a limited ability to fully represent community changes.

To further investigate the possible response of communities to nitrate-nitrogen I applied a multivariate ordination approach to test for indirect (unconstrained) and direct (constrained) gradients (Lepš & Šmilauer 2003a). Unconstrained ordinations separate communities which can be related with biotic metrics and environmental variables (Lepš & Šmilauer 2003a). In contrast, constrained ordinations display communities based on specifically selected environmental variables (Lepš & Šmilauer 2003a). Chapter 3 incorporates a combination of these techniques to test if nitrate-nitrogen is driving invertebrate composition, and uses these results to detect possible indicator taxa. Therefore, this analysis builds on Chapter 2 to provide a more rigorous method for investigating the relationship between nitrate-nitrogen and invertebrate community composition.

Relating invertebrate gradients to environmental variables and biotic metrics

Unconstrained ordination has been used frequently to investigate relationships between environmental variables and invertebrate community composition (Quinn & Hickey 1990; Hall et al. 2001; Brabec et al. 2004; Braccia & Voshell 2007; Greenwood et al. 2012). Braccia & Voshell (2007) investigated the invertebrate response with increased cattle grazing in Virginia, USA, and found changes in taxa community composition were associated with declines in biotic metrics and increased grazing intensity. Furthermore, Brabec et al (2004) investigated impacts of organic pollution on invertebrate communities using detrended correspondence analysis (DCA), which showed the first and second largest changes in community structure were correlated to organic enrichment and geological characteristics, respectively. In New Zealand, an investigation into the relationship between riparian condition and stream size on invertebrate communities documented two unconstrained axes were correlated to percent EPT taxa and QMCI scores (Greenwood et al. 2012). Subsequently, these axes provided responses in structural equation models that identified stream size and riparian condition as major determinants of community structure (Greenwood et al. 2012). These studies indicated one advantage of unconstrained ordination; that gradients of community composition produced were not constrained by variation only measured as environmental variables. Thus, unconstrained ordination can be used to determine the importance of different environmental drivers, rather than what community composition can be explained by selected observed environmental variables.

Invertebrate gradients explained by selected environmental variables

If changes in community composition across environmental gradients are observed, constrained ordination can test the importance of these environmental variables in driving community composition shifts given a clear and strong *a priori* hypothesis (Weckström, Korhola & Blom 1997; Harding et al. 1999; Johnson et al. 2007; Oksanen 2011). For example, a study of variables driving invertebrate communities in lowland and mountain streams across Europe found geographical position and ecoregion were the primary predictors of invertebrate composition over a broad spatial gradient (Johnson et al. 2007). Similarly, comparison between

different land-uses determined the most important environmental drivers of invertebrate composition using this technique in New Zealand (Harding et al. 1999; Duggan et al. 2002). Constrained ordination can also be used to assess the importance of a single environmental variable, whilst excluding other confounding variables, as a predictor of community change (Oksanen 2011). A study on Finnish lakes found diatom communities were strongly correlated with temperature alone, and as a result diatoms were identified as a useful tool to predict paleotemperatures from sediment-core diatom samples (Weckström et al. 1997). Similarly, a parallel study on the same lakes found chironomid subfossils were also a predictor of paleotemperature (Olander, Korhola & Blom 1997). These examples illustrate how constrained ordination was used to determine the importance of a single environmental variable in driving community change. Therefore, the results of this technique will identify the importance of nitrate-nitrogen in structuring invertebrate composition in the Canterbury Plains.

Aims of this chapter

My first aim was to determine environmental variables and biotic metrics that explain the greatest variation in benthic invertebrate community composition across a nitrate-nitrogen gradient in the Canterbury Plains streams. This was investigated using unconstrained ordination. The results could provide useful information on the most important environmental variables potentially driving community composition and the response of biotic metrics. A further aim was to investigate the singular effect of nitrate-nitrogen (by excluding confounding environmental variables) on community composition change using constrained ordination. This should indicate if nitrate-nitrogen is an important driver of community composition. A final aim investigated how individual taxa were related to unconstrained and constrained gradients to identify potential indicator taxa of related nitrate-nitrogen invertebrate community changes.

Methods

Unconstrained ordination

Detrended correspondence analysis (DCA) was used to identify shifts in community composition to produce relationships between unconstrained ecological assemblages of sites (Hill & Gauch 1980). The DCA was conducted with the decorana function in the community ecology package, Vegan of the open source statistics software R (v3.1) (Dixon 2003; Oksanen 2011). The decorana function incorporates detrending of gradient axes; i.e., rescaling axes to reduce packing of sites at gradient extremes and reduce the weight of rare species that have a high influence on ordination results (Oksanen 2011).

Initially, a DCA was conducted for all 41-field sites using presence/absence and abundance data. However, two sites (4 and 6) were considered outliers due to their exceptionally low channel stability and high fine sediments (site 4) and high discharge (site 6). An important criterion through my survey was to use sites with as few confounding variables as possible. Therefore, they were removed from the analysis. The binary presence/absence dataset was not transformed prior to analysis. The abundance dataset was converted to relative abundance as was an implicit assumption with DCA (Lepš & Šmilauer 2003b). The relative abundance data was then square root transformed to reduce the influence of many rare and few abundant taxa in the dataset (i.e., the abundance data contained many taxa only relatively abundant in a few sites) (Lepš & Šmilauer 2003a).

The relationships between community composition gradients and biotic metrics and environmental variables were tested using Pearson correlations. The positive or negative sign of correlations are only meaningful and comparable within and not between presence/absence and abundance data. To display the relationship between environmental variables and community composition gradients, environmental variables were overlayed onto the ordination diagram as vectors (Dixon 2003; Oksanen 2011). Fitted vectors are arrows

that represent direction of most rapid change in the environmental variable (gradient direction), and correlation strength with the ordination as represented by arrow length (gradient strength) (Oksanen 2011). These environmental vectors were tested for significance on the ordination using Monte Carlo permutation tests (999 permutations), which also produce a goodness-of-fit R^2 statistic (Oksanen 2011). Mean nitrate-nitrogen was fitted as a surface (represented as contour lines) to predict values of mean nitrate-nitrogen across the ordination (Dixon 2003; Oksanen 2011). The significance of the surface fitted was also tested using Monte Carlo permutation tests (999 permutations) (Oksanen 2011).

The unconstrained ordination produces taxa scores representing the position of taxa on unconstrained ordination gradients. Taxa scores were compared with the community gradient correlated with mean nitrate-nitrogen. This indicated how taxa were associated with the nitrate-nitrogen gradient.

Constrained ordination

DCA was also used to determine biological turnover, or gradient length of the presence/absence and abundance benthic invertebrate data. The longest gradient length of each dataset is related to whether a linear or unimodel model best describes the community composition response for constrained ordination (Lepš & Šmilauer 2003b): a linear response was selected for the presence/absence dataset because the gradient length was < 3; a unimodel response was selected for the abundance dataset with a gradient length > 3 (Lepš & Šmilauer 2003b). Redundancy analysis (RDA) was used to test for linear and canonical correspondence analysis (CCA) unimodel community composition response. These analyses were conducted in R statistical software with the community ecology package, Vegan (Oksanen 2011).

The ordination model constrained community composition by mean nitrate-nitrogen after conditioning out (i.e., removing) the variation associated with confounding variables detected in the unconstrained ordination. Monte Carlo permutation tests (999 permutations) were performed to determine if the nitrate-nitrogen constraint explained community composition better than at random without the constraint (Oksanen 2011).

Hickey (2013) has proposed a number of nitrate-nitrogen bands. I compared these with the constrained gradient using class centroids and permutation (999 permutations) tests (Oksanen 2011).

The constrained ordination produces taxa scores representing the position of taxa on the constrained ordination gradient. These scores indicate what nitrate-nitrogen concentrations taxa were associated with. Since the Macroinvertebrate Community Index (MCI) taxa scores were derived from tolerances to organic pollution, I tested if these MCI scores were related to taxa scores using linear regression.

Results

Unconstrained community composition gradients

For the presence/absence DCA discharge and shading were negatively correlated with axis 1, while mean nitrate-nitrogen was positively correlated with axis 2 (Table 5). Most biotic metrics were negatively correlated to axis 1 except functional feeding group richness, which was positively correlated (Table 5). Vectors overlayed onto the DCA to display environmental gradient direction demonstrated a weak correlation with discharge (R^2 = 0.16, p = 0.034) and shading (R^2 = 0.20, p = 0.019), which followed an approximately perpendicular and opposite gradient of community composition compared to mean nitrate-nitrogen (R^2 = 0.28, p = 0.006), respectively (Figure 9a). Predicted mean nitrate-nitrogen plotted as contour lines was significant (R^2 = 0.24, F_9 = 1.34, $P < 0.001$)(Figure 9a).

The DCA on abundance data differed in that both shading and %EPT3 / (% Oligochaeta + % Chironomidae) were correlated to axis 2 (Table 5). Shading and %EPT3 / (% Oligochaeta + % Chironomidae) were negatively correlated and mean nitrate-nitrogen positively correlated with axis 2 (Table 5). The percent variation of community composition explained by each axis was not produced with this DCA technique. Environmental vectors overlayed onto the DCA plot showed mean nitrate-nitrogen (R^2 = 0.28, p = 0.002) and shading (R^2 = 0.20, p = 0.025) display-opposing gradients in the abundance dataset (Figure 9b). Contour lines of predicted nitrate-nitrogen was significant (R^2 = 0.20, F_9 = 1.026, $P < 0.007$) (Figure 9b).

Table 5: Pearson correlations between DCA community compositon axes, environmental variables and biotic metrics for 39 sites. %EPT2 = percent EPT excluding *Oxyethira*. %EPT3 / (O + C) = ratio of percent EPT3 (EPT3 is taxa excluding pollution tolerant *Oxyethira*, *Paroxyethira* and *Aoteapsyche*) to percent Oligochaeta + percent Chironomidae. %EPT3 / (M + CRUS) = ratio of percent EPT to percent Mollusca + percent Crustacea. FFG = Functional feeding group richness. MCI = Macroinvertebrate Community Index. Correlation coefficients scaled between -1 and 1 displayed outside the brackets. P-values are indicated inside brackets; significance level detected at $\alpha = 0.05$.

	Presence/absence		Abundance	
	DCA1	DCA2	DCA1	DCA2
Environmental variables				
Mean nitrate-nitrogen		0.48 (0.002)		0.42 (0.008)
Shading	-0.33 (0.042)			-0.46 (0.003)
Discharge	-0.38 (0.016)		0.35 (0.03)	
Biotic metrics				
% EPT richness	-0.72 (< 0.001)		0.65 (< 0 .001)	
MCI	-0.45 (0.004)		0.54 (< 0 .001)	
FFG richness	0.39 (0.014)		-0.42 (0 .008)	
%EPT2	-0.72 (< 0.001)		0.66 (< 0.001)	
%EPT3 / (O + C)	-0.38 (0.018)			-0.46 (0 .003)
%EPT3 / (M + CRUS)	-0.48 (0.002)		0.41 (0 .01)	

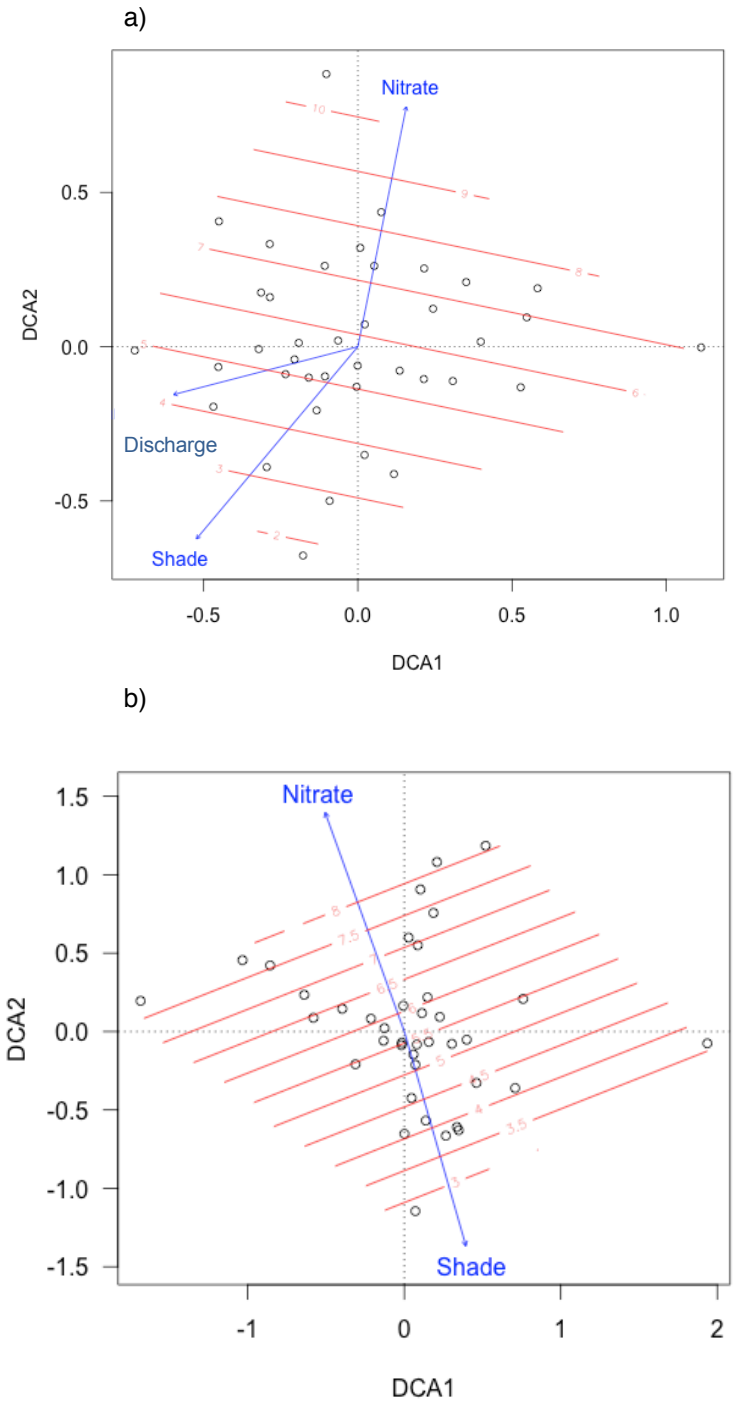

Figure 9: Unconstrained ordination (detrended correspondence analysis) tri-plot of; a) presence/absence and; b) abundance datasets of benthic invertebrate community composition. Arrows represent vectors of environmental variables associated with community composition change. Contours represent predicted values of mean nitrate-nitrogen.

Taxa related to nitrate-nitrogen correlated unconstrained community gradient

Taxa which occurred at all sites (e.g. *Deleatidium* and *Potamopyrgus*) exhibited scores close to zero on DCA axis 2, whereas taxa found at few sites occurred at either the left or right ends of axis 2 (Figure 10). A number of taxa were uncommon, however if we focus on taxa that occurred at ≥ 10 sites then three taxa; *Triplectides, Neurochorema* and *Oeconesus* were associated with lower nitrate-nitrogen (Figure 10). In contrast, taxa that occur ≥ 10 sites associated with higher mean nitrate-nitrogen were *Microvelia*, *Chironomus* and Elmidae larvae (Figure 10).

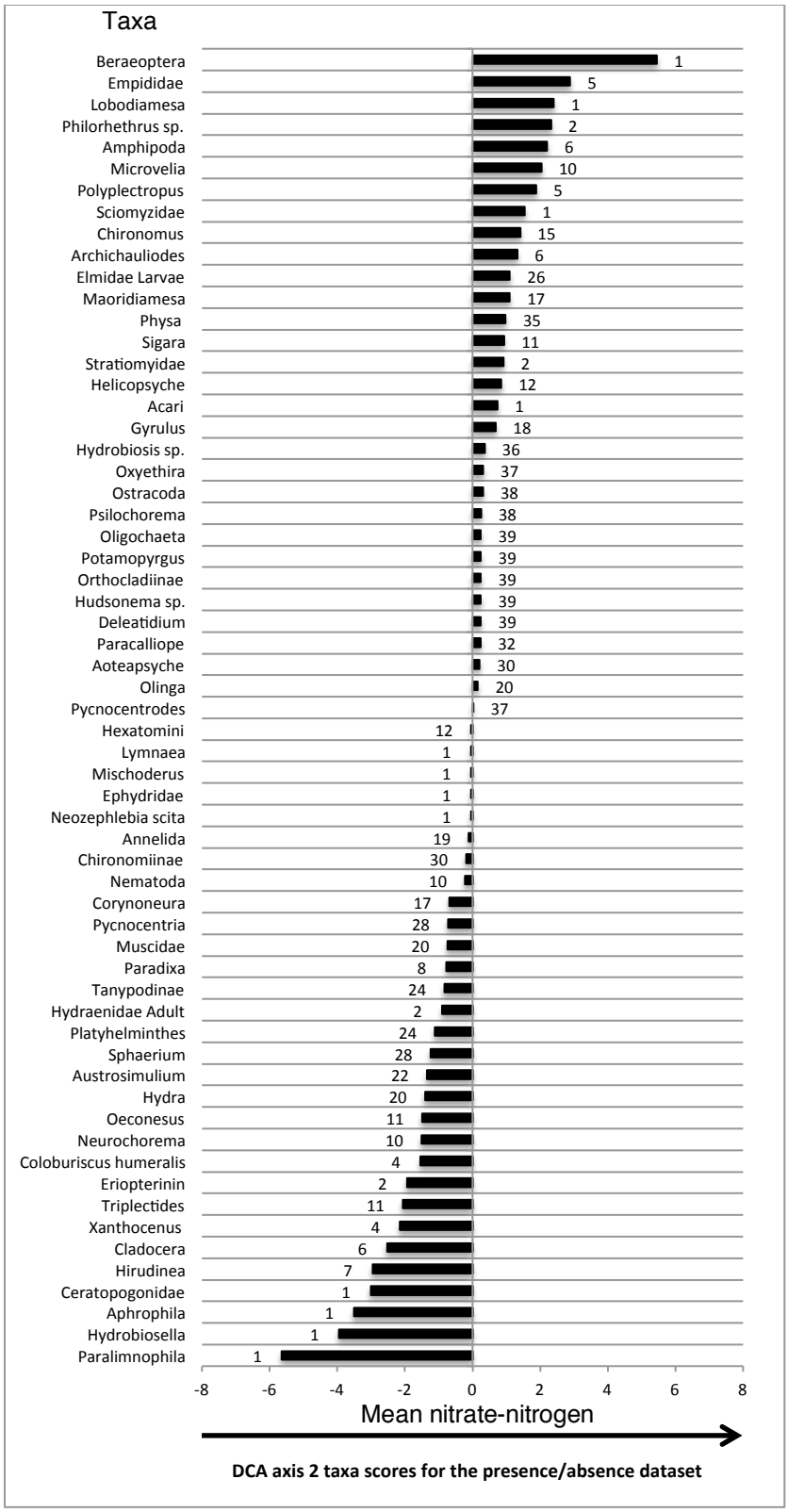

Figure 10: Presence/absence DCA axis 2 scores for 61 benthic invertebrate taxa correlated with mean nitrate-nitrogen gradient (Table 5). Frequency of occurrence of taxa indicated at the end of each bar.

Nitrate-nitrogen constrained ordination

The constrained presence/absence ordination showed a marginally non-significant gradient of macroinvertebrate community composition with mean nitrate-nitrogen (permutations = 7299, P = 0.057) (Figure 11a). The nitrate-nitrogen gradient explained 4% of total community composition after removing the variation associated with shading and discharge explaining 7.5%. The second, unconstrained axis explained 11% of community composition. Community composition also differed significantly between sites with differing levels of nitrate-nitrogen according to the recently proposed bands by Hickey (2013) (permutations = 999, R^2 = 0.38, P = 0.001) (Figure 11b). The greatest difference was displayed between the 99% and < 80% ecosystem protection bands, whilst the 95%, 90% and 80% groups were positioned similarly across the mean nitrate-nitrogen constrained axis (RDA1)(Figure 11). Similarly, a constrained ordination analysis for abundance did not show a significant relationship between the mean nitrate-nitrogen gradient and benthic macroinvertebrate community composition (permutations 999, P = 0.19).

Taxa related to constrained nitrate-nitrogen community gradient

In the constrained ordination, both rare (occurring only at 1 site) and abundant species were plotted more centrally and had lower taxa scores than in the unconstrained analysis (Figure 12). *Triplectides, Neurochorema* and *Oeconesus* were associated with lower and *Microvelia*, *Chironomus* and Elmidae larvae with higher mean nitrate-nitrogen (Figure 12). MCI scores attributed to the proposed sensitivity to organic pollution were not related to taxa scores determined by the constrained ordination analysis (R^2 = 0.03, F_{59} = 1.70, P =0.20).

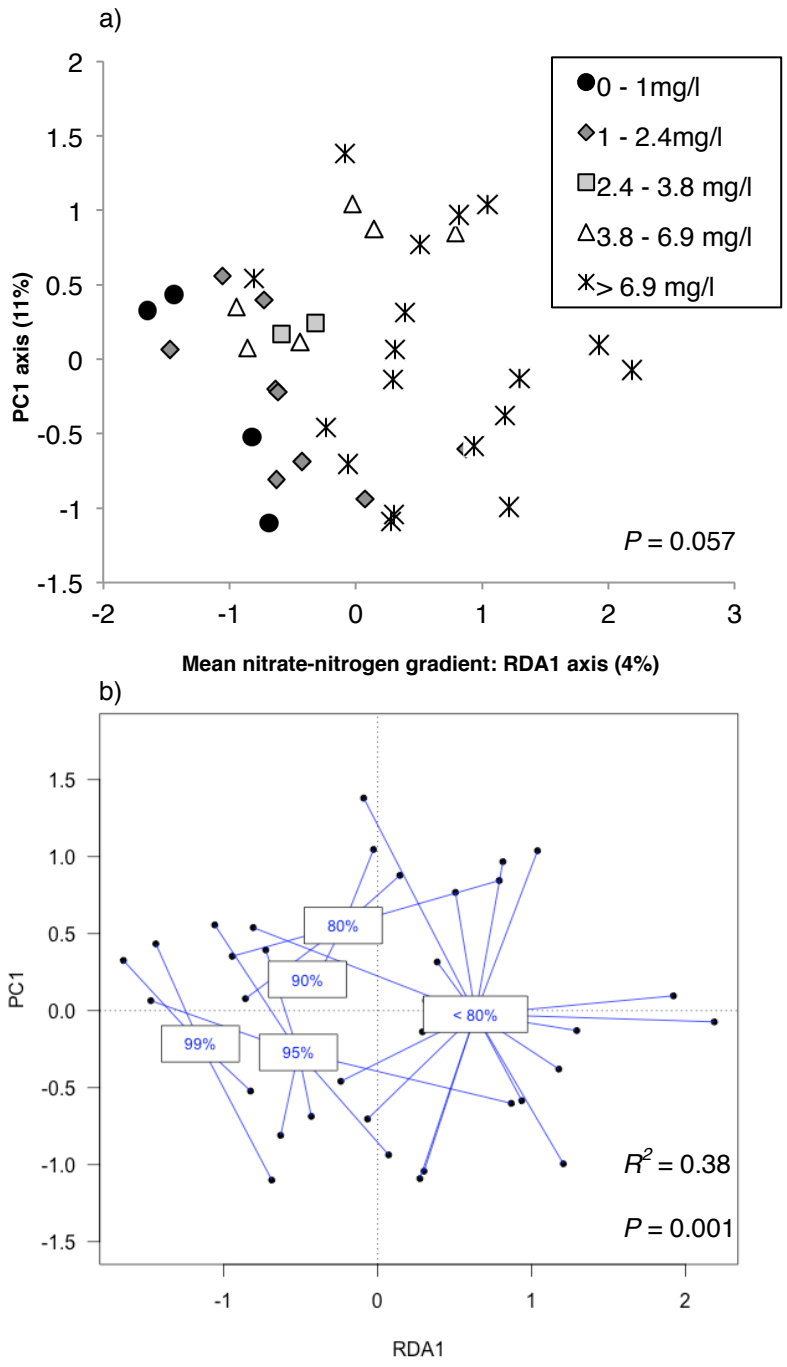

Figure 11: Ordination (redundancy analysis) of presence/absence data removing the effects of discharge and shading before constraining for variation in mean nitrate-nitrogen. A) Plot of community composition from 39 sites; RDA1 axis represents the mean nitrate-nitrogen gradient explaining 4% of community composition variation, with first unconstrained PC1 axis explaining 11%; *p-value* indicates mean nitrate-nitrogen is non-significant in the model. Legend indicates proposed Hickey (2013) nitrate-nitrogen bands. B) Plot of community composition from 39 sites with proposed Hickey (2013) nitrate-nitrogen bands represented as class centroids; *p-value* indicates a significant difference between centroids, and R^2 is a goodness-of-fit statistic.

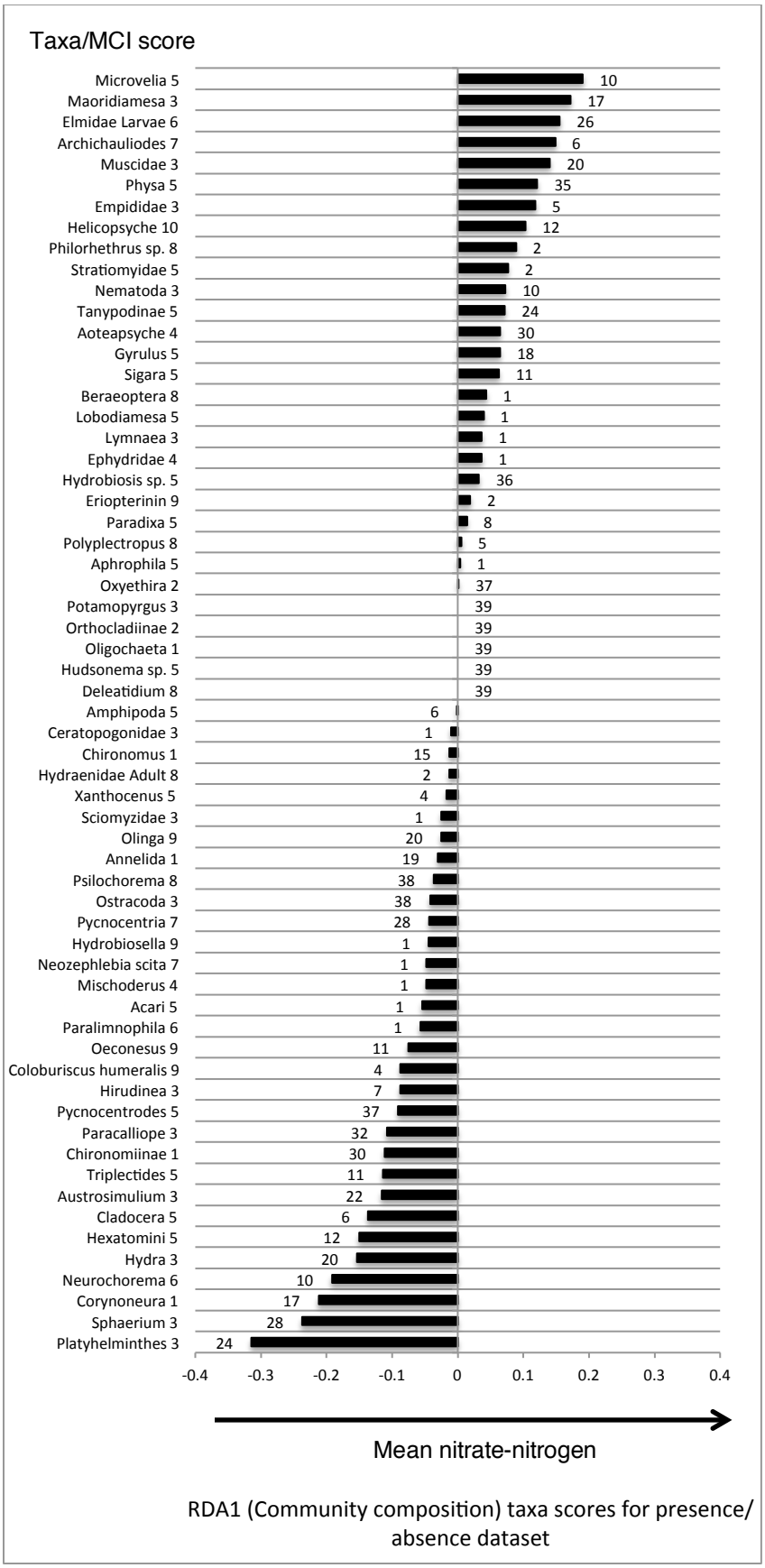

Figure 12: Community composition constrained by mean nitrate-nitrogen of 61 benthic invertebrate taxa for the presence/absence dataset. Frequency of occurrence of taxa indicated at the end of each bar. MCI score indicated after taxa name.

Discussion

Relationships between invertebrate composition gradients, biotic metrics and environmental variables

Unconstrained ordination analysis indicated discharge was the primary driver of changes in community composition across my sites. Similarly, Duggan et al. (2002) found discharge was responsible for most of the differences between invertebrate communities in Westland, New Zealand. In this chapter the biotic metrics associated with changing invertebrate communities were similar to those reported and discussed in Chapter 2, (i.e., increases in the proportion of sensitive taxa and decreases in functional feeding group richness).

In contrast, shade, which was not related to biotic metrics in Chapter 2, was correlated with changes in community composition. The importance of shade was higher in presence/absence than my abundance data. Sites with high shade were not associated with high nitrate-nitrogen sites, which may suggest shade mediates nitrate-nitrogen effects on invertebrate communities. For example, it has been suggested that stream communities may respond to shade via decreases in stream temperature (Parkyn et al. 2003) and light available for algal growth, which indirectly effects the dominance of particular invertebrate taxa at the patch scale (Quinn et al. 1997; Zimmermann & Death 2002). Furthermore, shade is associated with riparian buffer zones that may have reduced the transport of nitrate-nitrogen into waterways to affect stream communities (Storey & Cowley 1997), although shade may also decrease algal uptake of nitrate-nitrogen (Parkyn et al. 2003). Additionally, shade may provide an indirect pathway by altering algae community composition, shifting the food resources available and altering benthic invertebrate community structure (Thompson & Townsend 2004). Overall, nitrate-nitrogen and shade both limit algal growth (Hill & Knight 1988), therefore if algae composition is related to benthic invertebrate composition then limitation by one of these variables may mediate community composition shifts. Therefore, shade may be an important environmental variable to consider affecting stream health in the Canterbury Plains.

Mean nitrate-nitrogen was correlated to DCA axis 2, suggesting that nitrate-nitrogen was a major secondary driver. This finding is similar to those in Westland (Duggan et al. 2002) and Otago (Niyogi et al. 2007b), where nutrients were not correlated with the main community composition gradient.

I have not found any peer-reviewed New Zealand papers that have explicitly investigated the response of benthic invertebrate communities to nitrate-nitrogen. Thus, robust comparisons of my results against others were not possible. However, numerous studies investigating benthic communities in differing land-uses and nitrogen concentrations may be able to provide a partial comparison. For example, Liess et al (2012) suggested increased nutrients was partly responsible for shifts in invertebrate communities to species-poor assemblages across a dissolved inorganic nitrogen gradient of 0.029 – 2.57 mg/L. Furthermore, declines in EPT taxa abundance from native bush to agricultural land-use and increases in pollution tolerant taxa reflected by MCI and QMCI scores were associated with increased nitrate concentrations in Otago (Harding et al. 1999; Hall et al. 2001). Additionally, a longitudinal study in Otago found nutrients (up to 2mg/L) were positively related to land-use change, however sediment, which was also related to land-use, explained declines in biotic metrics (Niyogi et al. 2007a). These studies suggest nitrate-nitrogen can be correlated with declines in biotic invertebrate metrics, although nutrient gradients observed were much lower than recorded in this study, and confounding variables make it difficult to isolate potential nitrate-nitrogen effects.

Community composition gradient explained by nitrate-nitrogen

Direct gradient analysis did not show a significant gradient of community composition with nitrate. This result suggests nitrate-nitrogen does not explain a benthic invertebrate community composition gradient, although higher nitrate-nitrogen values were more likely to be associated with a greater shift in community composition. Sites with < 1mg/L of mean nitrate-nitrogen (representing the 99% ecosystem protection category (Hickey 2013)) were associated with slight shifts across the community composition gradient. Nation-wide studies comparing different land-uses and associated nitrogen concentrations indicated changes in structural metrics and ecosystem functions spanned a gradient of N from ~ 0.3 – 3.2 mg/L

(Clapcott et al. 2010; Clapcott et al. 2012). Similarly, shifts in benthic invertebrate communities have been documented in Otago (Riley et al. 2003; Niyogi et al. 2007) and Manawatu (Death 2014) with up to 1.9mg/L of nitrate-nitrogen. Therefore, benthic invertebrate communities may be expected to respond to as little as 1mg/L. Ideally I would have had more sites at these low nitrate-nitrogen concentrations in my study.

The nitrate-nitrogen bands proposed by Hickey (2013) were related to an invertebrate composition shift. The highest 99% and lowest < 80% ecosystem protection bands were more distinct than the 95%, 90% and 80% bands across the nitrate-nitrogen constrained gradient. This indicated the 95%, 90% and 80% bands that represented a 1 – 6.9 mg/L nitrate-nitrogen concentration range showed similar invertebrate composition. Therefore, nitrate-nitrogen concentrations < 1 and > 6.9mg/L may be important thresholds of invertebrate composition shifts.

Benthic invertebrate shifts and indicator taxa of nitrate-nitrogen

The only biotic metric that was correlated with nitrate-nitrogen was %EPT3 / (% Oligochaeta + % Chironomidae). This metric is the ratio of %EPT3 (EPT3 is EPT taxa excluding pollution tolerant *Oxyethira*, *Paroxyethira* and *Aoteapsyche*) to percent Oligochaeta + percent Chironomidae, which was designed to represent sediment stress by comparing sensitive to non-sensitive taxa (Meredith et al. 2000). However, since sediment was not a confounding variable in my study this metric is unlikely to be responding to sediment stress in these sites. Although %EPT3 / (% Oligochaeta + % Chironomidae) has not been specifically measured in studies of differences in land-use, shifts in the proportionate mean abundance of EPT taxa to Oligochaeta has been associated with higher nitrate-nitrogen (Quinn & Hickey 1990; Harding et al. 1999; Hall et al. 2001). However, it is unclear why other similar metrics (e.g., %EPT3 / (% Mollusca + % Crustacea) and %EPT2) were not correlated with community composition in this study. This may suggest %EPT3 / (% Oligochaeta + % Chironomidae) could be important for detecting nitrate-nitrogen effects. Overall, detection of nitrate-nitrogen effects is limited using biotic metrics in the Canterbury Plains, and

individual taxa associated with the nitrate-nitrogen gradient may provide more insights into community composition.

To identify potential indicator taxa benthic invertebrate taxa scores (from presence/absence data) were plotted to investigate how taxa were related to nitrate-nitrogen. Using the ordination scores to identify indicator taxa was difficult, because defining an appropriate occurrence frequency was subjective. For example, taxa that occurred in all sites had low scores, while rare taxa plotted closer to the extremes had high scores. However, rare taxa could occur by chance, or abundant taxa may not be sensitive to changes in community composition. Nonetheless, some common taxa, such as the predatory caddisflies *Triplectides*, *Neurochorema* and *Oeconesus*, which occurred at more than 10 sites, were related to unconstrained community composition scores and may be useful indicators of nitrate-nitrogen. Similarly, these taxa were also associated with lower nitrate-nitrogen concentrations in the constrained ordination. These three taxa were classified as potentially sensitive, according to the MCI system (5-9), and have the same predatory feeding mode, representing similar trophic niches. Liess et al (2012) noted the loss of predator invertebrate taxa with increasing dissolved inorganic nitrogen (0.03 – 2.5 mg/L). Overseas, the percent of predators in the invertebrate community has also been negatively correlated with organic pollution (Camargo, Alonso & De La Puente 2004). These studies indicate predators could be susceptible to nitrate-nitrogen, and therefore further investigation into tolerances of these taxa to nitrate-nitrogen is recommended.

The taxa *Microvelia*, *Chironomus* and Elmidae larvae were associated with higher mean nitrate-nitrogen in unconstrained and constrained ordination techniques, suggesting these taxa were tolerant to high nitrate-nitrogen concentrations. Elmidae larvae also indicated a similar response to the nitrate-nitrogen gradient in Westland, New Zealand (Duggan et al. 2002). Sensitive taxa (according to taxa MCI scores) of *Helicopsyche* and *Archichauliodes* (MCI = 10 and 7 respectively) were associated with higher mean nitrate-nitrogen concentrations, and *Deleatidium* (MCI = 8) was present in all sites. Consequently, MCI scores were not related to the mean nitrate-nitrogen constrained invertebrate gradient. The scores used in the MCI have

never been rigorously evaluated. They are based on expert opinion, based on Taranaki streams experiencing multiple environmental factors. My findings suggest that the MCI sensitivity scores may not be appropriate on the Canterbury Plains or, in fact, they may not truly represent tolerance to organically enriched water.

Hickey (2013), recommended four native macroinvertebrate species would be appropriate for nitrate-nitrogen toxicity testing to provide a more reliable statistical derivation of nitrate-nitrogen values: *Potamopyrgus antipodarum* (snail), *Parcalliope fluviatalus* (amphipod) *Sphaerium novaezelandiae* (bivalve)*, and Pycnocentria evecta* (caddisfly). These species were selected on criteria including known sensitivity to contaminants (amphipod and bivalve), being widespread in the environment (all except bivalve), and ecological importance (Hickey 2013). Of these taxa, *Potamopyrgus* occurred in all of my sites. This indicated nitrate-nitrogen was not a stressor that affected this snail. However, non-lethal effects on behaviour and reproduction of this species has been documented in response to nitrate-nitrogen (Alonso & Camargo 2013). Similarly, the caddisfly (*Pycnocentria*) was found in the majority of my sites suggesting no exclusion in response to nitrate-nitrogen. However, the bivalve was associated with low mean nitrate-nitrogen concentrations, potentially supporting sensitivity to nitrate-nitrogen. Finally, although amphipods were identified to Order they were only found in six sites, which suggested these taxa may not be a good indicator of nitrate-nitrogen on the Canterbury Plains. Overall, the macroinvertebrate species recommended by Hickey for nitrate-nitrogen toxicity testing with a non-lethal endpoint could provide useful information to support the proposed guidelines.

Conclusion

Discharge and shading were primary drivers and mean nitrate-nitrogen a secondary driver of benthic invertebrate community composition. Although similar, abundance differed from presence/absence data in that shade was correlated with the second largest change in community composition. Mean nitrate-nitrogen did not significantly explain the community composition for both datasets. I suggested sampling of more low mean nitrate-nitrogen sites would help investigate the shifts in community composition with respect to the Hickey's nitrate-nitrogen bands (2013). The majority of biotic metrics were not correlated to the second largest change in community composition with mean nitrate-nitrogen, therefore possibly indicating why no relationship was found in Chapter 2. Indicator taxa for nitrate-nitrogen could include selected predatory caddisfly species of *Triplectides*, *Neurochorema* and *Oeconesus* that have similar trophic niches. MCI scores were not related to community shifts in response to mean nitrate-nitrogen, which suggested assigned MCI sensitivity might not be appropriate on the Canterbury Plains. Overall, the community composition shift related to nitrate-nitrogen appears to be complex, and shifts of invertebrate taxa are difficult to detect on the Canterbury Plains.

Chapter 4: Discussion

Nitrate-nitrogen stress on biological function and community structure of freshwaters has been documented worldwide (Vitousek et al. 1997; Carpenter et al. 1998; Sala et al. 2000; Foley et al. 2005). However, the limited information on New Zealand stream fauna has raised concerns about the proposed nitrate-nitrogen guidelines aimed to protect ecosystem health (Hickey 2013). This is particularly important as national water quality "bottom lines" are currently in development (Ministry for the Environment 2013). These bottom lines are being proposed through the National Objectives Framework and will be brought into effect via the National Policy Statement on Freshwater Management. In effect, every waterbody in the country will be expected to meet these national bottom lines. My research included testing the proposed nitrate-nitrogen thresholds against common biotic metrics and community composition data.

In this Chapter I will discuss the implications of my findings for stream invertebrate community composition, structure, biotic metrics and algal mechanisms in response to nitrate-nitrogen. The application of knowledge for stream management and restoration, including importance of environmental variables, sensitivity of commonly used biotic indices to nitrate-nitrogen, and detection of indicator taxa will also be discussed. Overall, my research provided timely and important new data imperative for conserving stream life in the Canterbury Plains and New Zealand.

Effects of nitrate-nitrogen on stream ecological communities

The response of benthic invertebrate communities to nitrate-nitrogen have been indirectly studied through studies on land-use impacts in New Zealand (Quinn & Hickey 1990; Harding et al. 1999; Hall et al. 2001; Liess et al. 2012). As different land-uses have been associated with a multitude of stressors (e.g., sediment, flow and temperature (Harding et al. 1999; Matthaei et al. 2010)) benthic invertebrate changes can only be tentatively attributed to nitrate-nitrogen effects (Hall et al. 2001). In this regard my research was more targeted at possible nitrate-nitrogen effects by attempting to reduce (or account for) confounding variables, by focusing on similar sized lowland pasture streams, and by including streams that exhibited a much greater nitrate-nitrogen gradient (0.4 – 11.3mg/L) than other studies (e.g. 0.01 – 1.9 mg/L) (Death, unpublished data; Riley et al. 2003; Niyogi et al. 2007b). However, because of these differences, direct comparison with other studies is difficult. Nevertheless I believe my ability to identify any nitrate-nitrogen effects have been greatly improved by this research design.

Initially I found no correlation between nitrate-nitrogen and a number of common biotic metrics (Chapter 2). Subsequent ordinations showed that despite my attempts to reduce confounding factors, two parameters (discharge and shading) were still important in structuring benthic communities, and that nitrate-nitrogen effects were correlated to the second ordination axis (Chapter 3). These results suggest nitrate-nitrogen may not be the most important environmental variable affecting Canterbury streams. This may be partly due to stream benthic invertebrate communities on the Canterbury Plains which may have already been degraded and have experienced long-term nitrate-nitrogen (Greenwood et al. 2012, Chapter 2). Other studies, which have indicated possible nitrate-nitrogen effects, have tended to have much lower nitrate-nitrogen levels than in my study (Death, unpublished data; Riley et al. 2003; Niyogi et al. 2007b). Consequently, I suggest lower nitrate-nitrogen concentrations may produce a greater shift in communities with more sensitive taxa. Nitrate-nitrogen was important in structuring benthic invertebrate communities, although other environmental variables, e.g. sediment (Wagenhoff et al. 2011; Burdon, McIntosh & Harding 2013) probably provide a greater stressor in less targeted land-use studies (Liess et al. 2012). However, due

to a potential lag effect of nitrate-nitrogen concentrations in surface-waters (Stevenson et al. 2010), and increased dairy intensification (Pangborn & Woodford 2011) nitrate-nitrogen stress can be expected to increase in the future. Thus, shifts of invertebrate communities to more tolerant compositions may be observed, and these may become increasingly difficult to detect in the Canterbury Plains unless robust baseline data are collected now.

Some researchers have suggested that nitrate-nitrogen might cause a subsidy-stress relationship with invertebrate communities. This would occur where low nutrient concentrations would stimulate algal productivity and high concentrations would be detrimental by supporting excessive algal blooms (Riley et al. 2003; Niyogi et al. 2007b). If a unimodel relationship existed, it would provide a useful tool for detecting thresholds of community change (Niyogi et al. 2007b). In other research subsidy–stress relationships were observed across lower nitrate-nitrogen concentrations (Riley et al. 2003; Niyogi et al. 2007b; Wagenhoff et al. 2011), but my data potentially showed a linear "stress" indicating the unimodel "subsidy" may have been exceeded. This may support nitrate-nitrogen has already exceeded concentrations that might provide a subsidy benefit in the Canterbury Plains.

Despite my attempts to reduce confounding environmental variables, discharge and shade strongly influenced my communities and reduced my ability to detect any nitrate-nitrogen impacts on invertebrate communities (Chapter 3). Studies that investigated multiple stressors found nitrate-nitrogen impacts increased in low flow (Suren et al. 2003; Matthaei et al. 2010), and reduced discharge degraded sensitive invertebrate communities (Death et al. 2009). Therefore, since my high nitrate sites were not the same as my high discharge sites (Chapter 3), these results support the view that low flow may facilitate nitrate-nitrogen stress on benthic invertebrates (Matthaei et al. 2010). Furthermore, shade and nitrate-nitrogen displayed opposing gradients that suggest a mediation effect (Chapter 3). Interestingly, shade did not correlate with any biotic metrics (Chapter 2), however shifts in benthic invertebrate communities may have responded to algal community changes as a result of limited light (Quinn et al. 1997; Zimmermann & Death

2002; Thompson & Townsend 2004). This may be partly supported by my results which show increased food quality with higher nitrate-nitrogen as seen in gut contents of the common invertebrate grazer *Deleatidium* (Liess et al. 2012, Chapter 2). Therefore, nitrate-nitrogen effects on benthic invertebrate communities may be mediated by discharge and shading. My research did not investigate the mechanisms by which this may occur and this might provide a worthwhile focus for future research.

Applications for stream management and restoration

This research indicated discharge, shade and nitrate-nitrogen were important environmental variables for consideration in stream management, where discharge and shade potentially mediated the effects of nitrate-nitrogen. Thus management which focused on planting riparian buffers to improve stream shade cover (Parkyn et al. 2003) and maintenance of higher flows (Death et al. 2009) may help reduce nitrate-nitrogen effects and improve the restoration of benthic invertebrate communities in the Canterbury Plains (Greenwood et al. 2012). Moreover, my sites with nitrate-nitrogen concentrations above the 80% ecosystem protection band proposed by Hickey (2013) were correlated with a distinct shift in community composition, whereas sites below the highest 99% ecosystem protection did not change appreciably (Chapter 3). Therefore, the lower most and upper most bands at 1 mg/L and at 6.9 mg/L seem realistic, but intermediate nitrate-nitrogen bands were not supported by my data (Chapter 3).

Biotic metrics used as indicators of stream health for management were not sensitive to changes in nitrate-nitrogen except for %EPT3 / (% Oligochaeta + % Chironomidae) (Chapter 2 and 3). This metric is used by Environment Canterbury, however it is not used widely throughout the country. It was unclear why similar metrics were not also associated with the nitrate-nitrogen invertebrate gradient. Surprisingly, the MCI and QMCI scores in particular were not related to nitrate-nitrogen, which indicated these indices would be less useful in determining stream health in the Canterbury Plains. Changes in invertebrate community composition were the only responses related to mean nitrate-nitrogen, and may be more appropriate for assessing stream health.

Areas for future research

Several aspects of this research could fruitfully be expanded. My survey data could be strengthened by sampling invertebrate communities in more streams with < 1 mg/L of nitrate-nitrogen. This would provide a larger data set to detect any possible shift in invertebrate communities, and might provide better a comparison to other studies in New Zealand (Death, unpublished data; Riley et al. 2003; Niyogi et al. 2007b). Furthermore, the investigation of how shade, discharge and nitrate-nitrogen interact on invertebrate community composition would improve understanding of the importance of environmental stressors on the Canterbury Plains. Interactions between these variables may also provide a mechanism to improve stream health. Finally, ecotoxological trials on the three potentially sensitive predatory caddisfly taxa could test if these taxa were appropriate indicators of nitrate-nitrogen effects.

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